Expression of sphingosine kinase gene in the interactions between human gastric carcinoma cell and vascular endothelial cell

Juan Ren, Lei Dong, Cang-Bao Xu, Bo-Rong Pan

ABSTRACT

AIM: To study the interactions between human gastric carcinoma cell (HGCC) and human vascular endothelial cell (HVEC), and if the expression of sphingosine kinase (SPK) gene was involved in these interactions.

METHODS: The specific inhibitor to SPK, dimethyl sphingosine (DMS), was added acting on HGCC and HVEC, then the cell proliferation was measured by MTT. The conditioned medium (CM) of HGCC and HVEC were prepared. The CM of one kind of cell was added to the other kind of cell, and the cell proliferation was measured by MTT. After the action of CM, the cellular expression of SPK gene in mRNA level was detected with in situ hybridization (ISH).

RESULTS: DMS could almost completely inhibit the proliferation of HGCC and HVEC. The growth inhibitory rates could amount to 97.21%, 83.42%, respectively (P<0.01). The CM of HGCC could stimulate the growth of HVEC (27.0±0.01, P<0.01) while the CM of HVEC could inhibit the growth of HGCC (52.97±0.01 %, P<0.01). There was no significant change in the mRNA level of SPK gene in one kind of cell after the action of the CM of the other kind of cell.

CONCLUSION: SPK plays a key role in regulating the proliferation of HGCC and HVEC. There exist complicated interactions between HGCC and HVEC. HGCC can significantly stimulate the growth of HVEC while HVEC can significantly inhibit the growth of HGCC. The expression of SPK gene is not involved in the interactions.

INTRODUCTION

There exist many kinds of cells besides tumor cells in the solid neoplasm. These cells depend on each other and contribute together to the genesis, development, invasion and metastasis of tumor. The relations among all kinds of cells are very complicated. During the tumor angiogenesis and hematogenous metastasis, there exist complicated interactions between tumor cell(TC) and vascular endothelial cell(VEC)(1). In the pre-angiogenesis, how does TC induce VEC to establish the tumor vascular system? On the other hand, the interactions between the two cells play a role in the tumor hematogenous metastasis. There exist some complicated mechanisms in these processes. The study of tumor angiogenesis mainly focuses on the interactions among the vascular component cells while the study of tumor metastasis mainly focuses on the interactions between TC and its surrounding stroma. Seldom does anyone notice the interactions between TC and VEC. To better understand some mechanisms in human gastric carcinoma angiogenesis and hematogenous metastasis, we selected human gastric carcinoma cell (HGCC) and human vascular endothelial cell(HVEC) to study the interactions between HGCC and HVEC and some mechanisms involved in these actions. Sphingosine kinase(SPK) is a newly found important kinase in regulating many biological functions of most kinds of cells. SPK can induce the synthesis of extracellular transmitter and intracellular second messenger, sphingosine-1-phosphate (SPP). To determine whether SPK took part in regulating the proliferation of HGCC and HVEC, the specific inhibitor to SPK, dimethyl sphingosine(DMS), was acted on the two cells (2). The present study aims to probe into the interrelationships between HGCC and HVEC and if the expression of SPK gene was involved in these effects. The cell proliferation and the expression of SPK mRNA were measured after the action of the conditioned medium(CM) of the other kind of cell.

MATERIALS AND METHODS

Materials
Cell line HGCC line SGC7901 and HVEC line Eahy926 were employed.

Methods
Conditioned medium(CM) preparation, the ways of the actions of CM and DMS Cells in different confluent states were washed twice with PBS, then 3 mL culture medium was added to the cells. The medium what was taken as CM was collected after different periods. Cells were placed in serum free medium for growth arresting. After the growth of HGCC and HVEC was arrested for 24 h and 6 h respectively, the CM of the other kind of cell was added to the cell. The cell proliferation was measured by MTT after different cultivating periods. The cell without the action of CM was taken as control. The action way of DMS was as same as the way of CM. The cell without the action of DMS was taken as control. 

MTT (methyl tetrazolium colorimetry) 20 µl MTT solution (5 g/L) was added to 200 µl medium in each well of 96 well plate. 4h later, the supernant was discarded, 150 µl DMSO was added in. After the crystal was dissolved completely, absorption spectrum(A) was measured at 490 nm in the enzyme linked immunosorbent assay meter. The inhibitory rate of cell proliferation was calculated by:

\[ \text{Inhibitory rate} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100\% \]
proliferation = [1 - (the mean A of experimental group / The mean of control group ) ] ×100%

**Detecting the expression of SPK gene** After the action of CM of the other kind of cell, the cellular expression of SPK gene was detected by *in situ* hybridization (*ISH*) for mRNA level. There has been no antibody to SPK available up to now, so that it is impossible to detect the level of SPK protein. Probe specific to SPK mRNA was designed with the software “Primer 3”. The sequence of SPK probe is 5’ ATA TAC CAA GTA GGG GCA TTC ATA CTC 3’; Probe labeling and *ISH* were carried on according to the manual of the Dig Oligonucleotide Tailing Kit and Dig Detection Kit (*Boehringer Mannheim, Germany*) respectively. PBS was substituted for anti-Dig-Ap as negative control. The sections were analyzed for A value in the image analysis apparatus.

**Statistical analysis**

*t* test was used to compare the means.

**RESULTS**

**Effects of DMS on the proliferation of HGCC and HVEC**

DMS could almost completely inhibit the proliferation of HGCC and HVEC. The growth inhibitory rates could amount to 97.21 % and 83.42 % respectively (*P*<0.01 vs control). DMS could produce effects in 1 µmol/L and 3 h later. The inhibitory rate was related to the dose and action periods. 10 µmol/L DMS inhibited the proliferation of HGCC significantly, and the effect was increasing by leaps and bounds when the dose was from 3.5 µmol/L to 5 µmol/L or the action period was from 24 h to 48 h. 25 µmol/L DMS inhibited the growth of HVEC significantly, and the effect was increasing by leaps and bounds when the dose was from 10 µmol/L to 15 µmol/L or the action period was from 48 h to 72 h (Table 1, 2).

**Table 1** Dose-effect of DMS on HGCC and HVEC for 48 h (*n* =8, *x*±*s*)

| DMS dose/ (µmol/L) | Inhibitory rate of cell proliferation(%) |
|--------------------|----------------------------------------|
|                    | HGCC   | HVEC   |
| 0(control)         | 0      | 0      |
| 1                  | 12.33a | 5.61a  |
| 2                  | 17.53a | 11.11a |
| 2.5                | 31.31a | 17.77a |
| 5                  | 54.68a | 18.41a |
| 7.5                | 64.34b | 19.82b |
| 10                 | 80.69b | 31.02b |
| 15                 | 89.04b | 65.76b |
| 20                 | 92.67b | 76.02b |
| 25                 | 95.91b | 82.32b |
| 30                 | 97.21b | 83.42b |

*P*<0.01 vs control.

**Table 2** Time-effect of 10 µmol/L DMS on HGCC and HVEC (*n* =8, *x*±*s*)

| DMS action periods/ h | Inhibitory rate of cell proliferation(%) |
|-----------------------|----------------------------------------|
|                       | HGCC   | HVEC   |
| 0(control)            | 0      | 0      |
| 3                     | 9.11b  | 5.18b  |
| 6                     | 13.46c | 7.05c  |
| 12                    | 20.18d | 11.12d |
| 24                    | 41.98e | 19.52e |
| 48                    | 80.68f | 31.02f |
| 72                    | 93.55g | 56.44g |

*P*<0.01 vs control.

**Figure 1** Expression of SPK mRNA in HVEC after the action of the CM of HGCC. a: SPK mRNA in HVEC without the action of CM of HGCC (Control) (*in situ* hybridization); b: SPK mRNA in HVEC after the action of CM of HGCC (*in situ* hybridization)

**Figure 2** Expression of SPK mRNA in HGCC after the action of the CM of HVEC. a: SPK mRNA in HGCC without the action of CM of HVEC (Control) (*in situ* hybridization); b: SPK mRNA in HGCC after the action of CM of HVEC (*in situ* hybridization)
Effect of conditioned medium of gastric carcinoma cell on vascular endothelial cell

The conditioned medium of HGCC could stimulate the proliferation of HVEC (*P<0.05 vs no-CM action group) significantly. The stimulation effect was related to the different cell confluent states, different preparing periods, different volume fractions and different action periods (Table 3,4).

Effect of conditioned medium of vascular endothelial cell on gastric carcinoma cell

The conditioned medium of HVEC could inhibit the proliferation of HGCC significantly (*P<0.01 vs no-CM action group). The inhibitory effect was related to the different cell confluent states and different volume fractions (Table 5).

Table 3 Different dose-time-effects of CMs of subconfluent and confluent HGCC on HVEC (n=8, ±s)

| CM volume fraction | 100%CM | 80%CM | 50%CM | 30%CM | 10%CM |
|--------------------|--------|-------|-------|-------|-------|
| t / h cell confluent state | subconfluent HVEC | subconfluent HVEC | subconfluent HVEC | subconfluent HVEC | subconfluent HVEC |
| 24 | 2.38±0.01a 1.29±0.00a 2.70±0.01a 1.55±0.02a 2.21±0.01a 1.49±0.02a 2.10±0.03a 1.44±0.01a 1.92±0.02a 1.11±0.01a |
| 48 | 1.60±0.01a 1.22±0.01a 1.54±0.01a 1.33±0.02a 1.38±0.02a 1.23±0.02a 1.37±0.02a 1.21±0.01a 1.31±0.01a 1.15±0.02a |
| 72 | 0.89±0.00 0.85±0.00 0.99±0.01 1.03±0.01 1.07±0.01 1.11±0.01 1.27±0.01 1.01±0.00 1.01±0.00 1.15±0.01 |

‘P <0.05 vs No-CM action group.

Table 4 Different dose-time-effect of CMs of different preparing periods of confluent HGCC on HVEC (n=8, ±s)

| CM volume fraction | 100%CM | 80%CM | 50%CM | 30%CM | 10%CM |
|--------------------|--------|-------|-------|-------|-------|
| t / h Preparing periods | 24hCM | 48hCM | 24hCM | 48hCM | 24hCM | 48hCM | 24hCM | 48hCM | 24hCM | 48hCM |
| 24 | 1.29±0.01 1.38±0.01 1.55±0.02 1.39±0.01 1.49±0.01 1.38±0.01 1.44±0.02 1.30±0.01 1.11±0.01 1.00±0.01 |
| 48 | 1.22±0.01 1.11±0.02 1.33±0.01 1.14±0.02 1.23±0.01 1.03±0.01 1.21±0.01 1.03±0.01 1.15±0.01 1.02±0.01 |
| 72 | 0.85±0.01 0.78±0.00 0.99±0.01 0.92±0.00 1.07±0.02 1.14±0.01 1.27±0.01 1.20±0.01 1.15±0.01 1.16±0.01 |

‘P <0.05 vs No-CM action group.

DISCUSSION

In the human solid neoplasm, there exist many kinds of cells besides the tumor cell, such as: interstitial cell, immunocyte and vascular cell. They depend on each other to contribute to the tumor genesis, development, invasion and metastasis. There exist complicated interactions among these cells. During tumor angiogenesis and tumor hematogenous metastasis, both vascular endothelial cell (VEC) and tumor cell (TC) contribute to finishing the pathology processes. The interactions between VEC and TC in close coordination in establishing tumor vascular system and finishing hematogenous metastasis. There are complicated interactions between TC and VEC. In the pre-angiogenesis, how does TC induce VEC to establish the tumor vascular system? That is, how does TC influence the proliferation, degeneration, morphogenesis and functions of its neighboring VEC? Or, how does VEC influence these characters of its neighboring TC? These are all unclear. On the other hand, the interactions between two cells are involved in the tumor infiltration and hematogenous metastasis. Tumor vascular provides the passage for tumor infiltration and metastasis. The integrated vascular endothelium is the barrier.
to the tumor infiltration and metastasis. How do these two kinds of cells interact reciprocally to render tumor cell to adhere to and destroy the vascular endothelium to enter vascular lumen and damage it again to enter stroma? There exist some complicated mechanisms in this process. The study of tumor angiogenesis mainly focuses on the interactions among the vascular component cells while the study of tumor invasion and metastasis mainly focuses on the interactions between TC and surrounding stroma. Seldom has anyone noticed the interactions between TC and VEC. The relations between TC and VEC are not clear. Studies on this aspect are very few. Studies abroad always choose melanoma, glioblastoma, cephalo-cervical squamous carcinoma and hepatocellular carcinoma\(^1\)\(^{-}\)\(^8\), but not gastric carcinoma as the target research yet. And the results are also controversial. We have not found anyone who studies gastric carcinoma yet. There are lots of studies on gastric carcinoma\(^9\)\(^{-}\)\(^{19}\), but seldom in this aspect. To better understand some mechanisms in gastric carcinoma angiogenesis and hematogenous metastases, we select human gastric carcinoma cell (HGCC) and human vascular endothelial cell (HVEC) to study their interrelations and the mechanisms.

Results in this study showed that CMs of HGCC with different confluency, different preparing periods, different volume fractions and different action periods could all stimulate the growth of HVEC significantly. The activity of CM of subconfluent cell was stronger than that of CM of confluent cell. The activity of CM preparing for 24 h was stronger than that of CM preparing for 48 h. After the nutrition exhaustion was replenished, the more of the volume fraction, the stronger of the activity. This is consistent with the results of some studies. Someone found CM of bladder carcinoma stimulated the growth of HVEC. Others found that cephalo-cervical squamous carcinoma cell stimulated the growth of HVEC through secreting FGF and VEGF. But there were other contrary viewpoints. Zhao found bladder carcinoma cell inhibited the growth of HVEC through a 10-16bp fragment of tRNA. Albini found some kinds of TCs inhibited HVEC to form vascular through secreting IFN-\(\gamma\). There was also a neutral objection: TC has little effect on the proliferation of HVEC. Some researchers found that although TC had no effect on the growth of HVEC, TC could change morphology of HVEC or its sensitivity to TNF-\(\alpha\). We think these different results are due to different cell types. Our results also showed that CM of HVEC could inhibit the growth of HGCC significantly. The activity of CM of subconfluent cell was stronger than that of CM of confluent cell. After the nutrition exhaustion was replenished, the more of the volume fraction, the stronger of the activity. We do not know what this means exactly. Maybe in the tumor angiogenesis, this inhibition could prevent HGCC to occupy the place of vascular or participate into the vascularization. Then the stimulation of vascular system to the growth of gastric carcinoma is not through the direct interactions between HGCC and HVEC, but through the establishment of the vascular system that passes nutrition to TC and excretes its metabolism waste. Whether HVEC secretes some growth-inhibiting factors to inhibit the proliferation of HGCC or not needs further study.

Sphingosine 1-phosphate (SPP) is a newly found important extracellular transmitter and intracellular second messenger. SPP regulates many biological functions of most kinds of cells through taking part in several signal transduction pathways which include MAPK/ERK (mitogen activated protein kinase/extracellular regulating kinase, MAPK/ERK) pathway, CAMP signal transduction pathway, Ca\(^{2+}/\)CaM/Caldesmon, CaM pathway and phospholipase D pathway. The functions of SPP include: regulating the proliferation, morphology, migration, adhesion and apoptosis of cells, maintaining the structure of the endothelium and epithelium, regulating vasculogenesis, regulating the cardiovascular function, regulating the intracellular level of Ca\(^{2+}\) and K\(^{+}\), regulating tumor metastasis and so on\(^{20}\)\(^{-}\)\(^{39}\). The synthesis of SPP depends on the activation of sphingosine kinase (SPK). The activation of SPK is closely related to the survival and function of cells\(^{40}\)\(^{-}\)\(^{49}\). Lots of factors can activate SPK such as PDGF (platelet derived growth factor, PDGF) and Fc receptors. The concentrations of SPK in the plasma and serum are 200 nM, 500 nM respectively. There are several phosphorylated sites and combination sites for Ca\(^{2+}\) and CaM in the SPK sequence. The sequence of SPK is highly conservative from yeast, protozoan to mammal. The activity of SPK can be detected in the cytoplasm and cell membrane. The functions of SPK are somewhat similar to the functions of PLC (phospholipase C, PLC). To define the role of SPK in regulating the proliferation of HGCC and HVEC, the specific inhibitor to SPK, DMS, was added to the two cells. Results showed that DMS could almost completely inhibit the proliferation of HGCC, HVEC and DMS could produce effects in 1 \(\mu\)mol /L and 3 h later. This illustrated that the regulation of DMS on the proliferation of two cells was prompt, sensitive and striking. Considering SPK was an important regulator to the growth of HGCC and HVEC, we studied the role of SPK in the interactions between these two cells. We found that the expression of SPK mRNA in one kind of cell with no significant change after the action of the CM of the other kind of cell. This showed that the expression of SPK gene didn’t involve in the interactions between two cells. Maybe the regulation of one cell on the other cell is through some other factors or pathways. It needs further study to define the other mechanisms involved in the interrelationships between HGCC and HVEC.

REFERENCES

1. Ren J, Dong L, Xu CB, Pan BR, Li MZ. Interactions between the human gastric carcinoma cell and vascular endothelial cell. Shijie Huan ren Xue jia Zhi 2001; 9: 1254-1260

2. Ren J, Dong L, Xu CB. Role of sphingosine-1-phosphate in regulating the proliferation of human gastric carcinoma cell and vascular endothelial cell. Xi' an Yi Xue Xue bao 2003; 6:527-531

3. Von BC, Hayen W, Hartmann A, Mueller KW, Allolio B, Nehls V. Endothelial capillaries chemotactically attract tumor cells. J Pathol 2001; 193:367-376

4. Okamoto H, Ohigashi H, Nakamori S, Ishikawa O, Imaoka S, Mukai M, Kusama T, Fuji H. Reciprocal functions of liver tumor cells and endothelial cells. Involvement of endothelial cell migration and tumor cell proliferation at a primary site in distant metastasis. Eur Surg Res 2000; 32: 374-379

5. Moreno A, Villar ML, Camara C, Luque R, Cespon C, Gonzalez-PP, Roy G, Loper JJ. Interleukin-6 dimers produced by endothelial cells inhibit apoptosis of B-chronic lymphocytic leukemia cells. Blood 2001; 97:242-249

6. Brandvoit K A, Neiman P, Ruddell A. Angiogenesis is an early event in the generation of myc-induced lymphomas. Oncogene 2000; 19: 2780-2785

7. De BES, Rosati S, Jacobs S, Kamps WA, Vellenga E. Increased bone marrow vascularization in patients with acute myeloid leukaemia: a possible role for vascular endothelial growth factor. Br J Haematol 2001; 113:296-304

8. Hewett PW. Identification of tumor-induced changes in endothelial cell surface protein expression. In vitro
model. Int J Biochem Cell Biol 2001; 33:325-335

9 Luo J, Guo P, Matsuda K, Truong N, Lee A, Chun C, Cheng SY, Kore M. Pancreatic cancer cell-derived vascular endothelial growth factor is biologically active in vitro and enhances tumorigenicity in vivo. Int J Cancer 2001;92:361-369

10 Witt C, J, Gabel SP, Meisinger J. Interrelationship between protein phosphatase-2A and cytoskeletal architecture during the endothelial cell response to soluble products produced by human head and neck cancer. Otalaryngol Head Neck Surg 2000;122:721-727

11 Kuroda K, Miyata K, Tsutsumi Y. Preferential activity of wild-type and mutant tumor necrosis factor-α against tumorderived endothelial like cells. Jpn J Cancer Res 2000; 91:59-67

12 Shemirani B. Head and Neck squamous cell carcinoma lines produce biologically active angiogenic factors. Oral Oncol 2000; 36: 61-66

13 Beierle EA, Strande LF, Berger AC, Chen MK. VEGF is upregulated in a neuroblastoma and hematopoietic cell model. J Surg Res 2001;97:34-40

14 Vidal Vanaclocha F, Fantuzzi G, Mendoza L. IL-1β regulated IL-1β dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Nat Acad Sci USA 2000; 97:734-739

15 Aoki K, Kamonari M, Yudoh K, Ohmori K, Yasuda T, Kimura T. Effects of vascular endothelial growth factor and E-selectin on angiogenesis in the murine metastatic RCT sarcoma. Tumour Biol 2001;22:239-246

16 Liu W, Davis DW, Ramirez K, McConkey DJ, Ellis LM. Endothelial cell apoptosis is inhibited by a soluble factor secreted by human colon cancer cells. Int J Cancer 2001;92:26-30

17 Albini A, Marchihone C, Del Grosso F. Inhibition of angiogenesis and vascular tumor growth by interferon-α secreted by human colon cancer cells. Int J Cancer 2000; 87:1381-1393

18 Kamada H, Tsutsumi Y, Kihira T. In vitro remodeling of tumor vascular endothelial cells using conditioned medium from various tumor cells and their sensitivity to TNF-α. Biochem Biophys Res Commun 2000; 268:809-813

19 Wu K, Zhao Y, Liu BH, Li Y, Liu F, Guo J, Yu WP. RRR-α-tocopheryl succinate inhibits human gastric cancer SGC-7901 cell growth by inducing apoptosis and DNA synthesis arrest. World J Gastroenterol 2002;8:26-30

20 Wang X, Lan M, Shi YQ, Lu J, Zhong YX, Wu HP, Zai HH, Ding J, Wu KC, Pan BR, Jin JP, Fan DM. Differential display of vincristine-resistance-related genes in gastric cancer SGC7901 cell. World J Gastroenterol 2002;8:54-59

21 Yao YL, Xu B, Song YG, Zhang WD. Overexpression of cyclin E in Mongolian gerbil with Helicobacter pylori-induced gastric precancerous. World J Gastroenterol 2002;8:60-63

22 Yang SM, Fang DC, Luo YH, Lu R, Liu WW. Effect of antisense gene to human telomerase reverse transcriptase on telomerase activity and expression of apoptosis-associated gene. Shijih E Huan X Xiahua Z zhi 2002;10:149-152

23 Zheng ZH, Xu XJ, Qiu GR, Liu YX, Wang MX, Sun KL. E-cadherin gene mutation in precancerous condition early and advanced stage of gastric cancer. Shijih E Huan X Xiahua Z zhi 2002;10:153-156

24 Wang W, Luo HS, Yu BP. Expression of human telomerase reverse transcriptase gene and c-myc protein in gastric carcinogenesis. Shijih E Huan X Xiahua Z zhi 2002;10:258-261

25 Li JY, Yu JP, Luo HS, Yu BP, Huang JA. Effects of nonsteroidal anti-inflammatory drugs on the proliferation and cyclooxygenase activity of gastric cancer cell line SGC7901. Shijih E Huan X Xiahua Z zhi 2002;10:262-265

26 Xue FB, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of H.pylori infection with gastric carcinoma. Meta analysis. World J Gastroenterol 2001;7:801-804

27 Liu DH, Zhang XY, Fan DM, Huang YQ, Zhang JS, Huang WQ, Zhang YQ, Huang QS, Ma WY, Chai YB, Jin M. Expression of vascular endothelial growth factor and its role in oncogenesis of human gastric carcinoma. World J Gastroenterol 2001;7:500-505

28 Cai L, Yu SZ, Zhang ZF. Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: A case-control study. World J Gastroenterol 2001;7:506-509

29 He XS, Su Q, Chen ZC, He XT, Long ZF, Ling H, Zhang LR. Expression, deletion and mutation of p16 gene in human gastric cancer. World J Gastroenterol 2001;7:515-521

30 Boguslawski G, Grigg JR, Welch C, Ciechanowicz S, Silva D, Kovala AT, McGlynn P, Brindley DN, Rhodes RA, English D. Migration of vascular smooth muscle cells induced by sphingosine 1-phosphate and related lipids: potential role in the angiogenic response. Exp Cell Res 2002; 274:264-274

31 Mora A, Sabio G, Risco AM, Cuenda A, Alonso JC, Soler G, Centeno F. Lithium blocks the PKB and GSK 3 dephosphorylation induced by ceramide through protein phosphatase-2A. Cell Signal 2002;14:557-562

32 Shekar S, Tumaney AW, Rao TJ, Rajasekharan R. Isolation of LysoPhosphatic Acid Phosphatase from Developing Peanut Cotyledons. Plant Physiol 2002;128:996-996

33 Broomhead JM, Ledoux DR, Bermudez AJ, Rottinghaus GE. Chronic effects of fumonisin B1 in broilers and turkeys fed dietary treatments to market age. Poult Sci 2002; 81:56-61

34 Melendez AJ, Allen JM. Phospholipase D and immune receptor signalling. Semin Immunol 2002; 14:49-55

35 Itakura A, Tanaka A, Aioi A, Tonogaito H, Matsuda H. Ceramide and sphingosine rapidly induce apoptosis of murine mast cells supported by interleukin-3 and stem cell factor. Exp Hematol 2002; 30:272-278

36 Han X. Characterization and direct quantitation of ceramide molecular species from lipid extracts of biological samples by electrospray ionization tandem mass spectrometry. Anal Biochem 2002; 302:199-212

37 Mei J, Holst LS, Landstrom TR, Holm C, Brindley D, Manganello V, Degenan E. C2- ceramides influences the expression and insulin-mediated regulation of cyclic nucleotide phosphodiesterase 3B and lipolysis in 3T3-L1 adipocytes. Diabetes 2002; 51:631-637

38 Maruyama W, Oya-Ito T, Shamoto-Nagai M, Osawa T, Naoi M. Glyceraldehyde-3-phosphate dehydrogenase is translocated into nuclei through Golgi apparatus during apoptosis induced by 6-hydroxydopamine in human dopaminergic SH-SY5Y cells. Neurosci Lett 2002;321:29-32

39 Ryu Y, Takuwa N, Sugimoto N, Sakurada S, Usui S, Okamoto H, Matsui O, Takuwa Y. Sphingosine-1-phosphate, a platelet-derived lysophosphatidic acid mediator, negatively regulates cellular Rac activity and cell migration in vascular smooth muscle cells. Circ Res 2002; 90:325-332

40 Melendez AJ, Allen JM. Phospholipase D and immune receptor signaling. Semin Immunol 2002; 14:49-55

41 Melendez AJ, Khaw A.K. Dichotomy of Ca2+-signals triggered by different phospholipid pathways in antigen stimulation of human mast cells. J Biol Chem 2002; [epub
ahead of print]
42 Chiu TY, Hwang HM, Chueh SH. Distinct effects of different calcium-mobilizing agents on cell death in NG108-15 neuroblastoma X glioma cells. Mol Pharmacol 2002; 61: 486-494
43 McCaig C, Perks CM, Holly JM. Signalling pathways involved in the direct effects of IGFBP-5 on breast epithelial cell attachment and survival. J Cell Biochem 2002; 84: 784-794
44 Vann LR, Payne SG, Edsall LC, Twitty S, Spiegel S, Milstien S. Involvement of sphingosine kinase in TNF-alpha stimulated tetrahydrobiopterin biosynthesis in C6 glioma cells. J Biol Chem 2002; [epub ahead of print]
45 Xia P, Wang L, Moretti PA, Albanese N, Chai F, Pitson SM, D'Andrea RJ, Gamble JR, Vadas MA. Sphingosine Kinase Interacts with TRAF2 and Dissects Tumor Necrosis Factor-alpha Signaling. J Biol Chem 2002; 277: 7996-8003
46 Ancellin N, Colmont C, Su J, Li Q, Mittereder N, Chae SS, Stefansson S, Liu G, Hla T. Extracellular export of sphingosine kinase-1 enzyme. Sphingosine 1-phosphate generation and the induction of angiogenic vascular maturation. J Biol Chem 2002; 277: 6667-6675
47 Alemany R, Kleuser B, Ruwisch L, Danneberg K, Lass H, Hashemi R, Spiegel S, Jakobs KH, Meyer zu Heringdorf D. Depolarisation induces rapid and transient formation of intracellular sphingosine-1-phosphate. FEBS Lett 2001; 509: 239-244
48 Pitson SM, Moretti PA, Zebol JR, Vadas MA, D'Andrea RJ, Wattenberg BW. A point mutant of human sphingosine kinase 1 with increased catalytic activity. FEBS Lett 2001; 509: 169-173
49 Rosenfeldt HM, Hobson JP, Maceyka M, Olivera A, Nava VE, Milstien S, Spiegel S. EDG-1 links the PDGF receptor to Src and focal adhesion kinase activation leading to lamellipodia formation and cell migration. FASEB J 2001; 15: 2649-2659

Edited by Zhao P