Principal Investigator: Kazuyuki Kitatani
Grant Title: Involvement of acid β-glucosidase (GBA1) in cancer metastasis.

Abstract

Objective: Sphingolipids characterized with sphingobase backbone are an essential class of membrane lipids. Sphingolipid metabolism involves multiple metabolic pathways such as de novo pathway, salvage pathway and sphingomyelinase pathway *etc.* and a number of enzymes are involved. Ceramide, a central sphingolipid in the metabolism, has been emerging as a bioactive lipid that serves as a regulatory molecule in anti-inflammatory responses, apoptosis, autophagy, and cell motility of cancer cells. Very recently, we discovered that ceramide limits phosphatidylinositol-3-kinase C2β-controlled cell motility in ovarian cancer and proposed that ceramide serves as a tumor metastasis suppressor. Considering those bioactivities of ceramides, down-regulation of anti-metastatic ceramides is thought to promote the metastatic potential. Possibly, significant alternations in expression of sphingolipid metabolizing enzymes affect metastatic potential by down- or up-regulating cellar levels of ceramides. In the present studies, we characterized highly metastatic ovarian cancer cells with ceramide synthesis and identified anti-metastatic ceramide-generating enzyme responsible for suppressing cell motility and metastatic potential of ovarian cancer.

Methods: To establish highly metastatic cancer cells, in vivo screening was conducted in nude mice. SKOV3 ovarian cancer cells were inoculated into peritoneal of five-week-old female nude mice. Mice were sacrificed at 4 weeks after inoculation, and then four metastatic nodules were collected and cultured to establish metastasis-prone ovarian cancer cells. Analysis of sphingolipids in lipid extracts was performed by liquid chromatography-tandem mass spectrometry. Acid-β-glucosidase (GBA1) proteins were detected by the Western blotting.

Results: To define roles of ceramide in ovarian cancer, we tested effects of ceramide liposomes on the growth of ovarian cancer cells. Treatment of ovarian cancer cells with ceramide liposomes decreased the number of living cells through necroptosis but not apoptosis. Mechanistically, dying SKOV3 ovarian cancer cells exhibit activation of pseudokinase mixed lineage kinase domain-like as evidenced by oligomerization, showing necroptotic characteristics. Moreover, systemic ceramide liposomes administration suppressed metastatic growth in an ovarian cancer cell xenograft model. Therefore, ceramide is suggested to serve as a tumor suppressive lipid. To characterize metastatic cancer cells, we established metastasis-prone SKOV3 cells (M1-1, M1-2, M1-3, M1-4). Those cells showed decreased ceramide and higher invasiveness compared to the parental SKOV3 cells. Importantly, GBA1 expression was upregulated in all metastasis-prone cells. Considering that GBA1 cleaves glucosylceramide to form ceramide, GBA1-ceramide pathway is thought to limit cell growth and metastasis in ovarian cancer cells. In order to assess the clinical relevance of GBA1 as a tumor suppressor in ovarian cancer, an online analysis using ovarian cancer microarray datasets was performed. Patients were divided into 2 groups according to the GBA1 mRNA expression, giving high and low expression groups. In individual groups, patients-matched survival periods were plotted to generate Kaplan-Meier curves. Expression of LARP4 mRNA in ovarian cancer tissues is positively correlated with patient prognosis. Taken together, GBA1 is thought to serve as a tumor suppressor in ovarian cancer.