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Novel effects of FTY720 on perinuclear reorganization of keratin network induced by sphingosylphosphorylcholine: Involvement of protein phosphatase 2A and G-protein-coupled receptor-12

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ABSTRACT

Sphingosylphosphorylcholine (SPC) evokes perinuclear reorganization of keratin 8 (K8) filaments and regulates the viscoelasticity of metastatic cancer cells leading to enhanced migration. Few studies have addressed the compounds modulating the viscoelasticity of metastatic cancer cells. We studied the effects of sphingosine (SPH), sphingosine 1-phosphate (S1P), FTY720 and FTY720-phosphate (FTY720P) on SPC-induced K8 phosphorylation and reorganization using Western blot and confocal microscopy, and also evaluated the elasticity of PANC-1 cells by atomic force microscopy.

FTY720, FTY720P, SPH, and S1P concentration-dependently inhibited SPC-evoked phosphorylation and reorganization of K8, and migration of PANC-1 cells. SPC triggered reduction and narrow distribution of elastic constant K and conversely, FTY720 blocked them. A common upstream regulator of JNK and ERK, protein phosphatase 2A (PP2A) expression was reduced by SPC, but was restored by FTY720 and FTY72P. Butyryl forskolin, a PP2A activator, suppressed SPC-induced K8 phosphorylation and okadaic acid, a PP2A inhibitor, induced K8 phosphorylation. Gene silencing of PP2A also led to K8 phosphorylation, reorganization and migration. We also investigated the involvement of GPR12, a high-affinity SPC receptor, in SPC-evoked keratin phosphorylation and reorganization. GPR12 siRNA suppressed the SPC-triggered phosphorylation and reorganization of K8. GPR12 overexpression stimulated keratin phosphorylation and reorganization even without SPC. FTY720 and FTY720P suppressed the GPR12-induced phosphorylation and reorganization of K8. The collective data indicates that FTY720 and FTY720P suppress SPC-induced phosphorylation and reorganization of K8 in PANC-1 cells by restoring the expression of PP2A via GPR12. These findings might be helpful in the development of compounds that modulate the viscoelasticity of metastatic cancer cells and various SPC actions.

1. Introduction

The cause of death in most (90%) cancer patients is the development of metastatic lesions at sites distant from that of the primary tumor (Mendoza and Khanna, 2009). Recent novel approaches to characterize metastastic cancer cells include cell elasticity and mechanic properties, in recognition of the clinical importance of viscoelasticity and cell stiffness (Cross et al., 2007; Stroka and Konstantopoulos, 2014). Metastatic cancer cells recovered from pleural fluids of patients with suspected lung, breast and pancreas cancer are more than 70% softer than the benign cells (Cross et al., 2007). The importance of cell elasticity or viscoelasticity has been reported for several metastatic cancer cell lines. For example, the sphingosylphosphorylcholine (SPC)-induced keratin 8 (K8) reorganization and regulation of viscoelasticity in epithelial pancreatic cancer cells and the resulting changes in the mechanical deformability of the cells are potential pathways to facilitate the easier migration and increased metastatic competence of pancreatic tumor cells (Beil et al., 2003, 2012; Kim et al., 2015; Park et al., 2011a).

SPC is a lysosphingolipid involved in many biological processes including proliferation, cell migration, and wound healing. Increased levels of SPC in the blood and malignant ascites of patients

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with ovarian cancer are indicative of its potential importance in cancer biology, particularly metastasis, given that SPC can stimulate PANC-1 cell migration (Beil et al., 2003).

K8 is a simple epithelial-specific intermediate filament protein (Pan et al., 2013; Toivola et al., 2015). Typically, K8 and K18 are co-expressed as a primary keratin pair in simple epithelial cells (Fortier et al., 2013). Their expression is maintained during malignant transformation until the tumor becomes invasive (Fortier et al., 2013). Keratin reorganization is involved in migration and invasion of cancer cells (Beil et al., 2003; Kim et al., 2015; Windoffer et al., 2011). K8 reorganization is mediated through phosphorylation of specific serine residues in K8 by mitogen-activated protein kinases (MAPK), such as extracellular signal-regulated kinase (ERK), p38, and c-jun N-terminal kinase (JNK) (Beil et al., 2003; Ku et al., 2002; Park et al., 2011a; Snider and Omary, 2014).

Fingolimod (FTY720) is a sphingosine-related molecule with immunomodulatory function, that has been approved as an oral treatment for relapsing forms of multiple sclerosis (Takasugi et al., 2013). SphK2-mediated FTY720-phosphate (FTY720P) functions as an agonist for S1P receptors; and because FTY720P is an effective S1P analog, FTY720 corresponds to sphingosine (SPH) (Fig. 1) (Vessey et al., 2013).

SPC-induced K8 phosphorylation and reorganization are very interesting phenomena however, few compounds capable of modulating these processes have been reported. Recently ethacrynic acid was reported to suppress SPC-induced phosphorylation and reorganization, and migration of cancer cells (Byun et al., 2013). We have been interested in discovering compounds that suppress SPC-induced K8 phosphorylation and reorganization in PANC-1 cells, which would modulate the viscoelasticity of metastatic cancer cells.

In this study, we showed that FTY720 suppresses SPC-induced phosphorylation, perinuclear reorganization, and changes of elastic constant K of K8, leading to the inhibition of the PANC-1 cell migration by restoring the expression of PP2A via GPR12.

2. Material and methods

2.1. Materials

SPC was obtained from Matreya (Pleasant Gap, PA, USA). Phosphospecific antibody to K8 Ser431 was purchased from Abcam (Cambridge, UK). Anti-PP2A antibody and peroxidase-labeled secondary antibodies were acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Alexa Fluor 594 goat anti-mouse antibody was obtained from Molecular Probes (Eugene, OR, USA).

2.2. Cell culture

The PANC-1 human pancreatic carcinoma cell line (CRL 1469) was obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine (2 mM), penicillin-streptomycin (10,000 IU/ml and 10,000 μg/ml, respectively), and sodium pyruvate (1 mM). PANC-1 cells were maintained in a medium containing 10% (v/v) fetal calf serum (FCS). The cells were incubated at 37 °C in a humidified atmosphere containing 10% CO_2. The cells were recovered by centrifugation (200 g, 4 °C, 10 min) washed twice in serum-free DMEM using the same centrifugation conditions, and incubated in serum-free DMEM 18 h before the respective experiments. FTY720 (Cayman, Ann Arbor, MI, USA) and FTY720P (Cayman, Ann Arbor, MI, USA) at 5 μM were co-treated with SPC, respectively.

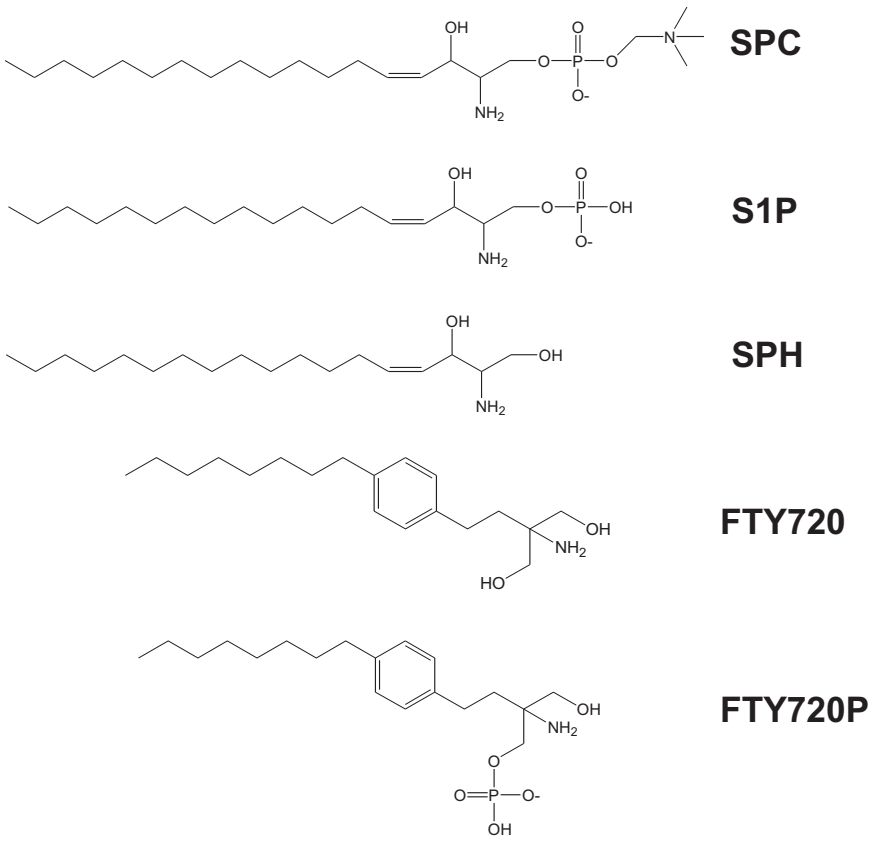

Fig. 1. Structure of SPH, S1P, FTY720, FTY720P, and SPC.