Neuronal chemorepellent Semaphorin 3E inhibits human airway smooth muscle cell proliferation and migration

Hesam Movassagh, MSc,[a] Lianyu Shan, MSc,[a] Andrew J. Halayko, PhD,[b] Michael Roth, PhD,[c] Michael Tamm, MD,[c] Jamila Chakir, PhD,[d] and Abdelilah S. Gounni, PhD[a] *Winnipeg, Manitoba, and Quebec City, Quebec, Canada, and Basel, Switzerland*

Background: Chronic airway diseases, including asthma, are characterized by increased airway smooth muscle (ASM) mass that is due in part to growth factor-mediated ASM cell proliferation and migration. However, the molecular mechanisms underlying these effects are not completely understood. Semaphorin 3E (Sema3E) has emerged as an essential mediator involved in cell migration, proliferation, and angiogenesis, although its role in ASM cell function is not investigated.
Objectives: We sought to determine the expression of Sema3E receptor, plexinD1, in human ASM cells (HASMCs); effect of Sema3E on basal and platelet-derived growth factor (PDGF)-induced proliferation and migration; and underlying signaling pathways.
Methods: Expression of plexinD1 in HASMCs was studied with RT-PCR, immunostaining, and flow cytometry. The effect of Sema3E on HASMC proliferation and migration was evaluated by 5-ethynyl-2′-deoxyuridine (EdU) incorporation, cell count, and Boyden chamber assay. Sema3E-mediated intracellular signaling was investigated with fluorescent microscopy, flow cytometry, Rac1 activation, and Western blot analysis.
Results: HASMCs from healthy persons expressed plexinD1 more than HASMCs from asthmatic patients. Sema3E increased plexinD1 expression in HASMCs from asthmatic patients. Recombinant Sema3E inhibited PDGF-mediated HASMC proliferation and migration, which was associated with F-actin depolymerization, suppression of PDGF-induced Rac1 guanosine triphosphatase activity, and Akt and extracellular signal-regulated kinase 1 and 2 phosphorylation. Bronchial biopsies from patients with mild asthma displayed immunoreactivity of plexinD1, suggesting the potential *in vivo* role of Sema3E–PlexinD1 axis in HASMC function.
Conclusion: This study provides the first evidence that Sema3E receptor is expressed and plays functional roles in HASMCs. Our data suggest a regulatory role of Sema3E in PDGF-mediated proliferation and migration, leading to downregulation of ASM remodeling. (J Allergy Clin Immunol 2014;133:560-7.)

Key words: *Airway smooth muscle cell, migration, platelet-derived growth factor, plexinD1, proliferation, semaphorin 3E*

Asthma is a complex and heterogeneous syndrome, which is characterized by reversible airway obstruction, inflammation, hyperresponsiveness, and remodeling.[1] Human airway smooth muscle cells (HASMCs) are a key cell type in asthma because of their ability to contract in response to inflammatory cell products. Because of their intrinsic plasticity, HASMCs also exhibit the capacity for multifunctional behavior and are actively involved in local inflammation and airway remodeling.[2]

Increased airway smooth muscle (ASM) mass, one of the hallmarks of airway remodeling, is commonly observed in patients with chronic airways diseases, including asthma and chronic obstructive pulmonary disease.[3-5] This pathology is believed to occur via multiple mechanisms such as cell proliferation and migration.[6,7] The increase in proliferation rate of HASMCs obtained from asthmatic patients compared with nonasthmatic subjects is remarkable.[8] HASMC proliferation is increased in response to allergen challenge, growth factors, and inflammatory mediators such as platelet-derived growth factor (PDGF)-BB,[9] epidermal growth factor (EGF),[10] and leukotriene B_4,[11] suggesting an important role of these factors in HASMC hyperplasia and airway remodeling. In addition, increased accumulation of ASM cells in asthma is not solely because of HASMC proliferation, and it might be partly attributable to migration of their progenitors from outside the muscle toward the lumen or immigration of proliferating cells within the muscle bundles.[12] Previous studies have reported promigratory effects of various growth factors and inflammatory mediators, including PDGF, TGF-β, and IL-8, on HASMCs.[13,14]

PDGF-BB modulates the contractile phenotype of HASMCs to a proliferative phenotype and stimulates their proliferation and migration *in vitro*.[14] Furthermore, Hitora et al[15] have shown mitogenic effect of this growth factor *in vivo*, whereby PDGF-BB overexpression in mice airways induced ASM hyperplasia and changed lung mechanics. However, the precise regulatory mechanisms underlying PDGF-induced HASMC proliferation and migration have remained elusive.

Semaphorins are a family of conserved-secreted and membrane-associated proteins originally discovered as axon guidance cues in neuronal development. More recently, their role in processes other than neuronal guidance was documented,

From the Departments of [a]Immunology and [b]Physiology, University of Manitoba, Winnipeg, Manitoba; [c]University Hospital Basel, University of Basel, Basel; and [d]Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie du Quebec, Université Laval, Quebec City, Quebec.

Supported by a grant from the Canadian Institutes of Health Research (CIHR) and Manitoba Health Research Council (MHRC). A.S.G. is supported by Manitoba Research Chair. H.M. is supported by a studentship from MHRC. M.R. is supported by the Swiss National Foundation (310030_130740/1).

Disclosure of potential conflict of interest: L. Shan has received a grant from the University of Manitoba. H. Movassagh has received a grant from the Manitoba Health Research Council (MHRC). A.S. Gounni has received grants from the Manitoba Institute of Child Health and the Manitoba Health Research Council, has patents that were submitted, but no financial gain was obtained in relationship to this work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 8, 2013; revised May 11, 2013; accepted for publication June 11, 2013.

Available online August 6, 2013.

Corresponding author: Abdelilah S. Gounni, PhD, Department of Immunology, Faculty of Medicine, University of Manitoba, 419 Apotex Center, 750 McDermot Ave, Winnipeg, Manitoba, R3E 0T5, Canada. E-mail: gounni@cc.umanitoba.ca.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.06.011

Abbreviations used

ASM: Airway smooth muscle
EdU: 5-Ethynyl-2′-deoxyuridine
EGF: Epidermal growth factor
ERK: Extracellular signal-regulated kinase
FACS: Fluorescence-activated cell sorting
GTPase: Guanosine triphosphatase
HASMC: Human airway smooth muscle cell
MAPK: Mitogen-activated protein kinase
MFI: Mean fluorescence intensity
PDGF: Platelet-derived growth factor
PI3K: Phosphatidylinositol 3-kinase
Rac1: Ras-related C3 botulinum toxin substrate 1
Sema3E: Semaphorin 3E
VEGFR2: Vascular endothelial growth factor receptor 2

which encompasses angiogenesis, differentiation, cell proliferation, and migration.[16] Semaphorin 3E (Sema3E) was previously described as an intrinsic mediator involved in axon path finding[17] and vascular patterning.[18] Sema3E interacts with its receptor, plexinD1, with high affinity and regulates migratory functions. Sema3E–plexinD1 axis has also emerged as a pivotal pathway in cell migration and angiogenesis in immune and endothelial contexts, respectively.[19,20] The antiangiogenic effect of Sema3E is mediated through inhibition of endothelial cell proliferation.[20] Therefore, it is a likely candidate to play a significant role in pathogenesis of airways diseases, where the mechanisms regulating these processes are impaired.

In this study we aimed to investigate the expression of Sema3E receptor on HASMCs and whether it affects basal and PDGF-induced HASMC proliferation and migration. We demonstrated that both normal and asthmatic HASMCs constitutively express Sema3E high-affinity receptor, plexinD1, importantly to a lesser extent in HASMCs from patients with asthma than from healthy subjects. Furthermore, Sema3E enhances plexinD1 surface expression in HASMCs from asthmatic patients. Sema3E significantly inhibited PDGF-BB–induced HASMC proliferation and migration via mechanisms that involve downactivation of Ras-related C3 botulinum toxin substrate 1 (Rac1) guanosine triphosphatase (GTPase), Akt, and extracellular signal-regulated kinases 1/2 (ERK1/2). PlexinD1 immunoreactivity was detected within the ASM bundle in bronchial sections of patients with mild allergic asthma. Our data suggest that Sema3E and its receptor are involved in the regulation of ASM remodeling in chronic airway diseases such as asthma.

METHODS

For details on the methods used in this study, please see this article's Methods section in the Online Repository at www.jacionline.org.

RESULTS

PlexinD1 is constitutively expressed by HASMCs *in vitro*

It has been previously shown that Sema3E binds plexinD1 with high affinity directly in neuronal and cardiovascular systems.[17,21] *In vitro* expression of Sema3E receptor in HASMCs was evaluated. As shown in Fig 1, *A*, mRNA for plexinD1 was expressed in primary HASMCs from 4 different donors. Human

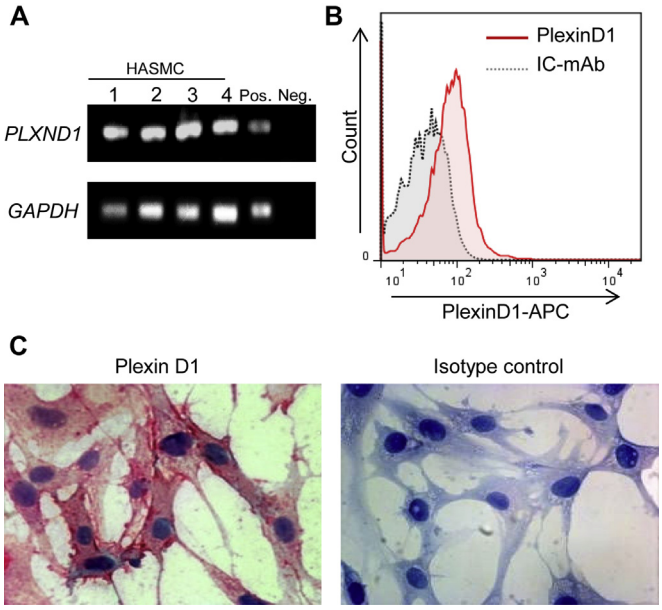

FIG 1. Expression of plexinD1 on HASMCs. Expression of plexinD1 on primary HASMCs was examined by RT-PCR with the use of specific primers **(A)**, flow cytometry **(B)**, and immunocytochemistry **(C)** by specific antibodies. Staining with isotype control antibody showed no immunoreactivity **(B** and **C)**. RNA and protein expression studies were performed on at least 3 different HASMCs under the same conditions. *APC*, Allophycocyanin; *GAPDH*, glyceraldehyde phosphate dehydrogenase gene; *IC*, isotype control; *PLXND1*, plexin D1 gene.

Universal Reference Total cDNA (Clontech, Calif) was used as a positive control in all RT-PCR experiments, and no cDNA was served as negative control tubes (see Table E1 in this article's Online Repository at www.jacionline.org). In parallel, flow cytometry with the use of specific mAb directed against human plexinD1 indicated its surface expression on HASMCs (Fig 1, *B*). We further confirmed expression of plexinD1 by performing immunocytochemistry on HASMCs (Fig 1, *C*).

PlexinD1 is expressed in allergic asthma with a differential surface expression between normal and asthmatic HASMCs

To examine whether plexinD1 is expressed *in vivo*, we performed immunostaining in bronchial tissue sections obtained from allergic asthmatic persons (n = 5). The clinical characteristics of the patients are provided in Table I. Fig 2, *A-C*, indicates that plexinD1 is expressed in ASM bundles and adjacent endothelium (red staining). Tissue sections stained with isotype antibody showed no immunoreactivity (Fig 2, *D*). Immunostaining of bronchial sections from healthy nonatopic subjects indicated plexinD1 expression in ASM bundles and airway endothelium (n = 5; data not shown) in a level comparable with asthmatic patients. However, fluorescence-activated cell sorting (FACS) analysis (Fig 2, *E* and *F*) found a significant decrease in plexinD1 surface expression on asthmatic versus normal bronchial HASMCs. Interestingly, treatment of HASMCs obtained from asthmatic patients with Sema3E, as a ligand, significantly increased surface expression of its receptor, plexinD1, to a level comparable with HASMCs from healthy donors (n = 3; $P < .05$; see Table E2 in this article's Online Repository at www.jacionline.org).