Histone Deacetylase 7 Silencing Alters Endothelial Cell Migration, a Key Step in Angiogenesis

Denis Mottet, Akeila Bellahcène, Sophie Pirotte, David Waltregny, Christophe Deroanne, Virginie Lamour, Rosette Lidereau, Vincent Castronovo

Abstract—Global inhibition of class I and II histone deacetylases (HDACs) impairs angiogenesis. Herein, we have undertaken the identification of the specific HDAC(s) with activity that is necessary for the development of blood vessels. Using small interfering RNAs, we observed that HDAC7 silencing in endothelial cells altered their morphology, their migration, and their capacity to form capillary tube-like structures in vitro but did not affect cell adhesion, proliferation, or apoptosis. Among several factors known to be involved in angiogenesis, platelet-derived growth factor-B (PDGF-B) and its receptor (PDGFR-β) were the most upregulated genes following HDAC7 silencing. We demonstrated that their increased expression induced by HDAC7 silencing was partially responsible for the inhibition of endothelial cell migration. In addition, we have also shown that treatment of endothelial cells with phorbol 12-myristate 13-acetate resulted in the exportation of HDAC7 out of the nucleus through a protein kinase C/protein kinase D activation pathway and induced, similarly to HDAC7 silencing, an increase in PDGF-B expression, as well as a partial inhibition of endothelial cell migration. Collectively, these data identified HDAC7 as a key modulator of endothelial cell migration and hence angiogenesis, at least in part, by regulating PDGF-B/PDGFR-β gene expression. Because angiogenesis is required for tumor progression, HDAC7 may represent a rational target for therapeutic intervention against cancer. (Circ Res. 2007;101:1237-1246.)

Key Words: angiogenesis ■ endothelial cells ■ HDAC ■ gene expression ■ PDGF-B

Angiogenesis is a crucial biologic event in physiologic conditions but also in many pathologic situations such as inflammation, tumor growth, and metastasis. This process depends on the activation/inactivation of genes associated with proliferation, adhesion, migration, and invasion of endothelial cells.1–2

Histone deacetylases (HDACs) constitute a family of enzymes that regulate gene transcription by modifying the acetylation level of histones and nonhistone proteins.3,4 Global inhibition of HDAC activity inhibits angiogenesis and tumor growth both in vitro and in vivo both by reducing expression of proangiogenic factors and enhancing expression of angiogenic inhibitors.5–10 At the time our study was initiated, no information was available regarding the identity of the HDAC(s) that could intervene in the control of angiogenesis. However, while we were preparing this report, a study was published that indicates a role for HDAC7 in the maintenance of vascular integrity.11

Here, we have applied a gene-silencing strategy using small interfering (si)RNAs efficiently targeting HDAC1 through HDAC7 in endothelial cells stimulated to form capillary-like structures. Our results indicate that HDAC7 is necessary for the assembly of endothelial cell in tube-like structures in vitro. We further show HDAC7 silencing causes cell shape changes and decreases endothelial cell migration, at least in part, through an upregulation of platelet-derived growth factor (PDGF)-B and its β receptor (PDGFR-β). Moreover, phorbol 12-myristate 13-acetate (PMA) induces the nuclear exportation of HDAC7 following protein kinase (PKC/PKD activation and stimulates PDGF-B production while partially inhibiting endothelial cell migration.

Materials and Methods

Reagents
PMA, ionomycin, and anti–β-actin antibody were from Sigma. Gö6983, Gö6976, and KN93 were purchased from Calbiochem. Anti-HDAC4 antibodies were from Active Motif or Santa Cruz Biotechnology. Anti-HDAC1, -HDAC3, -HDAC6, -Akt, and phospho (Ser473)-Akt antibodies were from Cell Signaling. Anti-HDAC2 and -HDAC7 antibodies were from Santa Cruz Biotechnology. Anti-HDAC5 antibodies were from Cell Signaling or Santa Cruz Biotechnology.

Original received June 19, 2006; resubmission received January 29, 2007; revised resubmission received September 20, 2007; accepted October 4, 2007.

From the Metastasis Research Laboratory (D.M., A.B., S.P., D.W., V.L., V.C.) and Laboratory of Connective Tissue Biology (C.D.), University of Liège, Belgium; and Laboratory of Oncology (P.L.), INSERM E0017, Centre René Huguenin and Institut National de la Santé et de la Recherche Médicale, U735, St Cloud, France.

Correspondence to Vincent Castronovo, MD, PhD, Head of the Metastasis Research Laboratory, Centre for Experimental Cancer Research, University of Liège, Pathology Building, B23, Level-1, B-4000 Liège, Belgium. E-mail vcastronovo@ulg.ac.be

© 2007 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.107.149377

1237
Cell Culture
Human umbilical vein endothelial cells (HUVECs) (passages 4 to 7) were isolated and maintained in culture as described previously.\(^\text{12}\)

Small Interfering RNA Transfection
siRNAs (Table I in the online data supplement at http://circres.ahajournals.org) were synthesized by Eurogentec and Dharmacon. Calcium phosphate-mediated transfections were performed as described previously.\(^\text{13}\)

Real-Time Quantitative RT-PCR
For the relative transcript expression analysis of a selection of angiogenic genes (supplemental Table II), total RNA extraction, cDNA synthesis, and PCR conditions were performed as described previously.\(^\text{14}\) Relative transcript expression levels of PDGF-A, PDGF-B, and PDGFR-\(\beta\) were assessed using TaqMan real time RT-PCR.

Immunocytochemistry
HUVECs were grown on glass coverslips. After fixation and permeabilization, cells were incubated with primary antibody and with an Alexa-488–conjugated secondary antibody (Molecular Probes) and mounted onto microscope slides. For nuclear counterstaining, cells were incubated with TOPRO-3 (Molecular Probes). Images were obtained with a Leica TCS SP laser-scanning confocal microscope (Leica). For F-actin staining, cells were fixed with...
paraformaldehyde and were incubated with rhodamine-conjugated phalloidin (Sigma).

**Western Blot Analysis**
Equal amounts of proteins were resolved by SDS-PAGE. The membranes were probed with primary antibodies, followed by horseradish peroxidase–conjugated secondary antibodies and developed using a chemiluminescence detection system. β-Actin was used as a control.

**PDGF Quantification Assay**
PDGF-AB/BB secretion in the medium was assayed by ELISA (Quantikine, R&D Systems) according to the instructions of the supplier.

**In Vitro Capillary Tube-Like Network Formation Assay on Type I Collagen Gel or Matrigel**
Endothelial capillary tube-like network formation was assessed using type I collagen gel matrix as described previously or using Matrigel (Chemicon) as instructed by the manufacturer.

**Scratch–Wound Assay**
Confluent HUVEC monolayers were scratched to create a wound. After wounding and 6 hours later, 2 different fields of each wound were photographed with a phase-contrast microscope. Measurements of the width of each wound were made in each experimental condition. At the start of the experiment, the wound size was measured and scored as 100%. After 6 hours, the width of the remaining wound was measured and the average percentage of wound closure was calculated.

**Motility Assay**
Chemotaxis and haptotaxis were assayed using the QCM Chemotaxis Cell Migration Assay and the QCM Collagen I Quantitative Haptotaxis Cell Migration Assay (Chemicon), respectively, as instructed by the manufacturer.

**Endothelial Cell Adhesion Assay**
HUVECs were incubated in vitronectin-, fibronectin-, gelatin-, laminin–, or type I collagen– precoated wells. Attached cells were stained with crystal violet, and the incorporated dye was measured by reading absorbance at 560 nm.

**Endothelial Cell Proliferation Assay**
After transfection, HUVECs were trypsinized and viable cells were grown for an additional time. Cells were harvested and sonicated in PBS. Fluorimetric DNA titration was performed as described previously and used as an indicator of cell density.

**Annexin V Apoptosis Analysis**
Adhesive cells were washed, trypsinized, and pooled with detached cells. After centrifugation and washing steps, the pellet was incubated in the presence of Annexin V-FLUOS. Propidium iodide was added before flow cytometric analysis using a FACSCalibur cytometer (BD Biosciences) and standard CellQuest software.

**Chromatin Immunoprecipitation Assay**
Chromatin immunoprecipitation assay was performed according to the procedure of the manufacturer (Upstate Biotechnology), with some modifications. PCR was performed with a pair of primers (5’-GGATCTTATGGAGCATTTTC-3’ and 5’-CCAAAGGATGCTGTGGTTGG-3’) designed to detect nucleotide sequences encompassing the human proximal PDGF-B promoter.

**Results**

**HDAC7 Is Necessary for Human Endothelial Cell Tubulogenesis In Vitro**
We examined the impact of HDAC silencing on the capacity of HUVECs to form capillary-like structures in vitro.

![Figure 2.](image)

**Figure 2.** HDAC7 silencing does not affect proliferation, apoptosis, or adhesion. A, The efficiency of HDAC7 silencing in mock-transfected (no siRNA) and HDAC7 siRNA– and scHDAC7 siRNA– transfected HUVECs was determined by Western blot 24 or 48 hours after transfection. At these times, HUVEC proliferation (B) and apoptosis (C) were assessed by fluorimetric DNA titration and Annexin V staining. Corresponding data are representative of 2 separate experiments, each measurement was performed in triplicate, and the results are presented as means ± SD. D, Assessment of cell adhesion to vitronectin (VN), fibronectin (FN), gelatin (GL), laminin (LM), and type I collagen (COL). The number of adherent cells was quantified as described in the online data supplement. Data are representative of 2 separate experiments, each measurement was performed in 6 replicates, and the results are presented as means ± SD.

HUVECs were transfected with siRNAs directed against HDAC1 to −7. The expression of each HDAC protein was reduced by the corresponding siRNA (Figure 1A). Interestingly, only HDAC7 silencing inhibited the spontaneous outgrowth of endothelial cells and their organization in capillary/tube-like structures between 2 type I collagen layers (Figure 1B). Such an inhibition was also observed when
Matrigel was used in the assay (Figure 1C), suggesting that the inability of HUVECs to form a capillary/tube-like network in response to HDAC7 inhibition may not be dependent on the type of matrix. HDAC7 siRNA did not affect the transcript expression levels of the other HDACs (Figure 1D), suggesting that HDAC7 silencing is specifically involved in the inhibition of tubulogenesis in vitro.

**HDAC7 Silencing Inhibits Migration but Not Proliferation, Apoptosis, or Adhesion of Endothelial Cells**

Angiogenesis involves multiple events, including endothelial cell proliferation, survival, and migration. To examine the role of HDAC7 in these events, HUVECs were transfected without siRNA, with HDAC7 siRNA, or with scHDAC7 siRNA. For each experiment, the suppression of HDAC7 protein expression was confirmed by Western blot (Figure 2A). As shown in Figure 2B and 2C, HDAC7 silencing did not affect cell proliferation or apoptosis. In addition, HDAC7 inhibition did not modulate endothelial cell adhesion to various components of the extracellular matrix (Figure 2D).

The impact of HDAC7 silencing on cell migration was first explored using the scratch–wound assay. As shown in Figure 3A, wound healing was significantly reduced in HDAC7-silenced cells. After wounding, only 10%±1.8% of the scratched area was covered by migrating endothelial cells, whereas 59%±1.5% and 55%±3.2% of the wound surface were covered by mock- or scHDAC7 siRNA–transfected HUVECs. Haptotaxis (C) and chemotaxis (D) of mock-, HDAC7 siRNA–, and scHDAC7 siRNA–transfected HUVECs to type I collagen were performed as described in the online data supplement.

![Figure 3](image-url)
cells, respectively (Figure 3B). To determine whether the inhibition of tubulogenesis between 2 collagen gels was related to the inhibition of migration, we investigated the effect of HDAC7 silencing on HUVEC migration in Boyden chambers coated with type I collagen substratum, which can provide both the motility stimulus to migrate (haptotaxis) and the substrate for cells to migrate in response to soluble chemoattractant gradient (chemotaxis). HDAC7 silencing reduced the ability of HUVECs to migrate on collagen I concentration gradient (chemotaxis) (Figure 3C), as well as toward type I collagen-coated chambers in response to chemoattractant gradient (chemotaxis) (Figure 3D). These results may at least partly explain how HDAC7 silencing prevents the formation capillary-like structures by HUVECs in type I collagen gel matrix.

**HDAC7 Silencing Increases PDGF-A/B mRNA As Well As PDGF-AB/BB Protein Secretion**

To investigate the mechanisms by which HDAC7 silencing alters endothelial cell migration and tubulogenesis, we screened changes in transcript expression levels of several angiogenic factors using real-time RT-PCR in HDAC7-silenced HUVECs (supplemental Table II). Interestingly, the PDGF-A and PDGF-B genes were found to be the most upregulated genes in response to HDAC7 silencing. PDGF-A/B mRNA overexpression was independently confirmed by real-time RT-PCR in mock-, HDAC7 siRNA-, and scHDAC7 siRNA–transfected HUVECs (Figure 4A).

PDGFs consist of a family of homo/heterodimers of disulfide-bonded A and B chains resulting in 3 isoforms, PDGF-AA, PDGF-AB, and PDGF-BB, synthesized and secreted by endothelial cells.20 The 3 isoforms have been reported to act as chemoattractants on endothelial cells.20 The PEFG-B isoform is considered to be the least potent of the 3 isoforms,21,22 and at high concentrations, PDGF-AB and PDGF-BB proteins may completely block cell migration.23–26 ELISA tests enabled confirmation that HUVEC transfection with HDAC7 siRNA induced a ≥2.5- to 3-fold induction of secretion of both PDGF-AB (896±41 pg/mL; PDGF-BB, 141±1 pg/mL) or with scHDAC7 siRNA (PDGF-AB, 314±34 pg/mL; PDGF-BB, 140±3.5 pg/mL) (Figure 4B).

**HDAC7 Silencing Inhibits Endothelial Cell Migration and Causes Cell Shape Change, at Least in Part, via PDGF-B Overexpression**

PDGF-B has been originally identified as a stimulating factor during angiogenesis, but excessive PDGF-B may also inhibit migration and tubulogenesis of endothelial cell.24,25 Thus, we examined whether the increased expression of PDGF-A/B induced by HDAC7 silencing participated in the inhibition of HUVEC migration. siRNAs directed against PDGF-A and PDGF-B transcripts were cotransfected with HDAC7 siRNA. In these experiments, GL3 and scHDAC7 siRNAs were used as controls. No cellular toxicity was associated with the double-siRNA transfection (data not shown). The siRNAs directed against PDGF-A and PDGF-B induced a downregulation of their mRNA when cotransfected with scHDAC7 siRNA and, more importantly, prevented in a specific manner the increase in PDGF-A and PDGF-B transcript expression induced by HDAC7 silencing (Figure 5A). In HUVECs transfected with scHDAC7 siRNA, no significant change in migration rates was observed when cells were depleted in PDGF-A or PDGF-B mRNA (Figure 5B). More importantly, HDAC7 silencing–mediated migration inhibition was partly relieved by cotransfection of the cells with a siRNA targeting PDGF-B. Such a reverse effect was not observed when HDAC7-silenced cells were cotransfected with a PDGF-A siRNA (Figure 5B), suggesting that the impaired migration of HDAC7-silenced HUVECs is attributable, at least partly, to an excessive production of PDGF-B. However, because recombinant PDGF-BB was not sufficient to mimic the effects of HDAC-7 inhibition on migration phenotype (online data supplement), it confirms that the inefficient migration of HDAC7-depleted HUVECs is partially triggered by PDGF-B overexpression and probably involved others genes modulated by HDAC7 inhibition.

High concentrations of PDGF-BB have been shown to inhibit cell migration by acting via the PDGF-β recep-
However, in HUVECs, the PDGFR-β mRNA was barely detectable (data not shown and elsewhere), but the expression of this gene was significantly increased in response to HDAC7 silencing (supplemental Table II), suggesting that excessive PDGF-BB might prevent migration only via action on endothelial cells expressing PDGFR-β following HDAC7 silencing. These considerations led us to investigate the involvement of HDAC7 silencing–induced PDGFR-β overexpression in the inhibition of endothelial cell migration. As shown in Figure 5C and 5D, HDAC7 silencing–mediated migration was partly relieved by cotransfection of the cells with a siRNA targeting PDGFR-β, suggesting that the impaired migration of HDAC7-silenced HUVEC migration is also attributable to an excessive production of PDGFR-β. These considerations might explain why the addition of recombinant PDGF-BB to untransfected endothelial cells (in extenso cells not expressing PDGFR-β) did not inhibit their migration because it would not possibly recapitulate the dynamic interplay between PDGF-B and its receptor in the context of HDAC7 silencing.

As shown in other cell types, PDGF-BB activates the PDGFR-β/phosphoinositide 3-kinase/Akt pathway, which contributes to the reorganization of the cytoskeleton and controls migration. However, at high concentrations of PDGF-BB, the PDGFR-β phosphorylation is transient, the downstream pathway is saturated at the level of phosphoino-
sitide 3-kinase and the kinetics of 3’-phosphoinositides production is limited with Akt activation closely following the level of 3’-phosphoinositides. With these considerations, we investigated the effect of PDGF-B overexpression induced by HDAC7 silencing on both endothelial cell cytoskeleton morphology and Akt activation. Interestingly, HDAC7 silencing was associated with substantial morphological alterations in HUVECs (Figure 5E, c) and in the distribution of the actin filaments (Figure 5E, h). These morphological alterations and changes in actin filaments organization were partly relieved by cotransfection of the cells with a siRNA targeting PDGF-B (Figure 5E, d and j), suggesting that the modified morphology induced by HDAC7 silencing is attributable, partly, to an excessive production of PDGF-B. Concerning Akt activation, we demonstrated that HDAC7 silencing inhibited its activation. This inhibition was partly relieved when cells were cotransfected with a siRNA targeting PDGF-B (Figure 5F). Collectively, these results led us to hypothesize that PDGF-B overexpression induced by HDAC7 silencing may alter the cytoskeleton because of a partial inhibition of phosphoinositide 3-kinase/Akt pathway.

A PMA-Activated PKC/PKD Pathway Promotes Nuclear Export of HDAC7 and PDGF-B Expression

Exportation of HDAC7 out of the nucleus through its phosphorylation via the activation of the Ca^{2+}/calmodulin-dependent kinase or PMA-PKC/PKD pathway is associated with a repression of its activity. In HUVECs, treatment with PMA, but not with ionomycin, promoted HDAC7 nuclear export (Figure 6A), suggesting that HDAC7 exportation out of the nucleus is dependent on the PMA-activated PKC/PKC pathway but independent from a calcium pathway. Because the PMA-activated PKC/PKD pathway has been shown to induce the nuclear export of HDAC5, we also examined whether other class II HDACs could be exported out of the nucleus in response to PMA. Only HDAC7, and not HDAC4 or HDAC5, was significantly exported out of the nucleus in response to PMA (Figure 6B). To confirm that PMA induces nuclear export of HDAC7 through a PKC/PKD-dependent pathway, we used G06983, a general inhibitor of PKCs, and G06976, an inhibitor that targets both calcium-dependent PKC isoforms and PKD. Both specific
inhibitors prevented HDAC7 nuclear export mediated by PMA. However, KN93, an inhibitor of the Ca\(^{2+}\)/calmodulin-dependent kinase pathway, had no effect (Figure 6C).

Because our results clearly show that HDAC7 is exported out of the nucleus in response to PMA and given that PMA has been described to upregulate PDGF-B transcription, we next sought to determine whether PMA could also inhibit endothelial cell migration through PDGF-B overexpression. HUVEC migration was assessed in response to PMA after transfection either with control GL3 or with PDGF-B siRNAs. PMA treatment of GL3 siRNA-transfected cells resulted in a strong migration inhibition concomitant with increased PDGF-B transcript expression (Figure 7B and 7C). More interestingly, in PDGF-B–depleted cells, this inhibition was not observed in response to PMA, suggesting that the blockade of PDGF-B overexpression abolishes PMA-induced inhibition of endothelial cell migration (Figure 7B). These results further underline the role of an excessive production of PDGF-BB in the inhibition of endothelial cell migration.

HDAC7 Silencing Does Not Modulate Histone H3 Acetylation Levels at the PDGF-B Promoter

Because HDACs are known to regulate gene transcription by modifying the acetylation level of histones, we evaluated the presence of acetylated histone 3 (H3) at the PDGF-B promoter in HDAC7-silenced HUVECs using chromatin immunoprecipitation assays. In HDAC7 siRNA-transfected HUVECs, H3 acetylation was not observed at the PDGF-B promoter (Figure 8A). We also investigated whether PMA-induced HDAC7 exportation from the nucleus would modify H3 acetylation levels at the PDGF-B promoter. PMA treatment did not influence acetylation levels of H3 associated with PDGF-B promoter (Figure 8B).

Discussion

In this study, we have shown that HDAC7 silencing in endothelial cells alters their morphology and motility and prevents their assembly in tube-like structures in vitro without affecting their adhesion, proliferation, or apoptosis. The mechanism by which HDAC7 silencing inhibits endothelial cell migration and disturbs cell morphology involves, at least in part, the stimulation of PDGF-B and PDGFR-\(\beta\) expression. This is strongly supported by the demonstration that when the expression of PDGF-B and PDGFR-\(\beta\) induced by HDAC7 silencing is prevented by the use of specific siRNA, the effect of HDAC7 silencing on endothelial cell migration is partially relieved. Although several studies have reported that PDGF-B induces differentiation of endothelial cells and changes their phenotype\(^{41,42}\) and that PDGF-BB has previously been shown to induce reorganization of cytoskeletal components in endothelial cells\(^{43-45}\) leading to endothelial dysfunction,\(^{45}\) further investigations are needed to completely delineate the molecular mechanisms linking endothelial cell cytoskeleton alterations, cell migration inhibition, and PDGF-B overexpression induced by HDAC7 silencing. Nevertheless, we have demonstrated that PDGF-B overexpression...
induced by HDAC7 silencing is associated with reduced phosphorylation of Akt, a kinase well described to regulate many cellular functions, such as migration and cell shape. Seeking to understand how HDAC7 silencing upregulates PDGF-B expression, we found that this HDAC does not regulate PDGF-B through a histone-H3 acetylation-dependent mechanism. However, the regulation of the PDGF-B expression has already been described to be HDAC dependent because the basal PDGF-B promoter has been shown to be activated by trichostatin A. These findings led us to hypothesize that HDAC7 might control PDGF-B expression through an indirect mechanism, for example, by regulating the activity of transcription factors (eg, Sp-1) known to regulate PDGF-B expression. Preliminary data have indicated that HDAC7 appears to control Sp-1 DNA-binding activity (data not shown). Moreover, because previous reports have also demonstrated that trichostatin A treatment increases PDGF-β promoter activity through a CCAAT motif\(^{17}\) encompassing 2 binding sites for the Sp-1 transcription factor, which has been described to play a key role in PDGF-β transcription,\(^{48}\) it raise the possibility that HDAC7 may regulate the expression of both PDGF-B and PDGF-β via a common mechanism possibly involving Sp-1, but this hypothesis needs to be confirmed.

If HDAC7 controls angiogenesis partially through negative PDGF-B and PDGF-β regulation, we cannot rule out that HDAC7 silencing–mediated abnormal angiogenesis might result from an overall dynamic imbalance between pro- and antiangiogenic factors. Our transcriptomic screening has revealed that HDAC7 may regulate the expression of several other angiogenic genes (supplemental Table II), and it has been reported recently that HDAC7 maintains the structural integrity of the endothelium by regulating matrix metalloproteinase-10 gene expression through the transcriptional factor myocyte-enhancer factor-2,\(^{11}\) substantiating that HDAC7 controls angiogenesis through the regulation of multiple angiogenic genes.

The nucleocytoplasmic shuttling of class II HDACs through phosphorylation is a well-described mechanism that regulates their transcriptional repression activities.\(^{49,50}\) In thymocytes, PMA-activated PKC/PKD pathway induces the phosphorylation of HDAC7, leading to its export from the nucleus, with subsequent loss of its repression activity on Nur77 promoter.\(^{34,36}\) Similarly, in PMA-stimulated endothelial cells, we demonstrated that the selective exportation of HDAC7 out of the nucleus is associated with the overexpression of PDGF-B and that the similarity between HDAC7 silencing through RNA interference and its exportation from the nucleus following PMA activation in terms of PDGF-B overexpression is also correlated with reduced endothelial cell migration, suggesting that the upregulation of PDGF-B acts as negative regulator of endothelial cell migration.

In summary, among the 7 first members of the HDAC family, HDAC7 silencing is sufficient to inhibit angiogenesis in vitro. This HDAC therefore may be a potential target for the development of antiangiogenic treatments.

Sources of Funding
This work was supported by grants from European FP6 (STROMA and METABRE), the National Fund for Scientific Research (Belgium), the Centre Anti-Cancéreux près de l’Université de Liège, the Fonds Léon Frédéricq, TELEVIE and Interuniversity Attraction Pole Program–Belgian Science Policy (IAP 5/31). A.B., C.D., and D.W. are Research Associates at the National Fund for Scientific Research (Belgium), V.L. and S.P. are NFSR National Fund for Scientific Research fellows.

Disclosures
None.

References