

# A novel angiogenic peptide, $\Delta$ ADT: A truncated adrenotensin peptide revealed by secretory peptidome analysis of human retinal pericytes

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**Summary** Retinal pericytes play an important role in the maintenance of retinal microvascular homeostasis. We performed a secretory peptidome of primary human retinal pericytes. Using liquid chromatography-tandem mass spectrometry analysis in the culture medium of retinal pericytes, we identified 256 peptides derived from 114 proteins, and identified a novel partial fragment Leu163-His183 (termed  $\Delta$ ADT) of adrenotensin (ADT). To elucidate the role of  $\Delta$ ADT as a soluble mediator of pericyte-endothelial cell interactions, we investigated the bioactivity of  $\Delta$ ADT in human retinal microvascular endothelial cells (HRMVECs). The cell proliferation assay indicated that the proliferation of HRMVECs was promoted by ADT or  $\Delta$ ADT. Moreover,  $\Delta$ ADT had a greater growth promoting effect than ADT in HRMVECs and induced migration and tube formation of HRMVECs. We also observed actin reorganization and that the levels of phosphorylated focal adhesion kinase in  $\Delta$ ADT stimulated HRMVECs. These results showed that  $\Delta$ ADT induces profound actin reorganization and increases the levels of phosphorylated focal adhesion kinase. Collectively, our study showed that  $\Delta$ ADT has an angiogenic activity, and suggested that  $\Delta$ ADT is a novel angiogenic peptide.

**Keywords:** Retinal pericytes, secreted peptides, peptidomics, angiogenic peptide, adrenotensin

## 1. Introduction

Retinal capillaries are composed of microvascular endothelial cells, pericytes, and basement membrane. Retinal pericytes fulfill important roles in a range of functions, including angiogenesis, vessel stabilization, endothelial cell regulation, and maintenance of the blood-retinal barrier. *In vitro* pericyte and endothelial cell co-culture experiments have suggested that pericytes inhibit endothelial cell proliferation in a contact-dependent manner (1) and may increase the barrier function established by endothelial cells (2), a process to which transforming growth factor  $\beta$ 1 (3)

and angiotensin 1 (Ang1) (4) contribute. Angiogenesis involves endothelial cell proliferation, migration, and tube formation. The pericytes provide paracrine signals that promote vascular stability. Ang1-Tie2 signaling of endothelial cells is important for angiogenesis and vascular remodeling and has been suggested to be required for vascular stabilization and maturation (5). The Tie2 receptor is specifically found on endothelial cells, whereas Ang1 is mainly expressed in pericytes. This finding demonstrated that pericyte-endothelial cell interactions are mediated by soluble factors. However, the soluble factors mediating pericyte-endothelial cell interactions are not completely understood. Further studies are required to clarify the mechanism of the interaction.

Peptidomics has been advocated for the comprehensive study of peptides cleaved from precursor proteins by endogenous proteases (6,7). These naturally occurring peptides are beyond the reach of current proteomics examining trypsin-digested peptides, and should be analyzed in their native forms. Unlike proteomics, peptidomics has the potential to uncover processing sites

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of precursor proteins (8).

In the present study, we searched for novel peptides that may play a role in endothelial homeostasis and as a novel molecular target for retinal vascular diseases. We identified secretory peptides in human retinal pericytes using liquid chromatography-tandem mass spectrometry (LC-MS/MS) based peptidomics, and identified an angiogenic partial fragment of adrenotensin (Leu163-His183, termed  $\Delta$ ADT).

## 2. Materials and Methods

### 2.1. Cell cultures

Primary human retinal pericytes (passage 2) and primary human retinal microvascular endothelial cells (HRMVECs, passage 3) were purchased from the Applied Cell Biology Research Institute (Kirkland, WA), respectively. Pericytes were cultured on poly-D-lysine-coated cell culture dishes in CSC Complete Serum-Free Medium Kit with RocketFuel. HRMVECs were cultured on type I collagen-coated cell culture dishes in EGM-2 MV medium (EBM-2 supplemented with EGM-2 MV SingleQuots; Lonza, Walkersville, MD). These cells were used at passages 7-9.

### 2.2. Preparation of the fraction of secreted peptides from retinal pericytes

Semi-confluent retinal pericytes were washed twice with pre-warmed Dulbecco's Modified Eagle Medium (DMEM)/Ham's F-12 medium without phenol red. Subsequently, the cells were cultured in the same medium for 24 h. After incubation, the medium was collected and filtered through a 0.22  $\mu$ m filter. The solution was then acidified to pH 3.0 with trifluoroacetic acid (TFA) and subjected to a C18 solid phase extraction cartridge (Empore 10 mm/6 mL; 3M Company, St. Paul, MN) equilibrated with 2% acetonitrile and 0.1% TFA (buffer A). The cartridge was washed with buffer A, and the fraction of crude peptides was eluted with 80% acetonitrile and 0.1% TFA (buffer B). The eluent was evaporated in a vacuum concentrator and dissolved in 100  $\mu$ L of 30% acetonitrile and 0.1% TFA (buffer C). The sample was separated by gel filtration column chromatography (TSKgel G2000SWXL; Tosoh, Tokyo, Japan) in buffer C at a flow rate of 1 mL/min. Corresponding peptide fractions (from the 8th to 11th fractions) were collected, combined, and then loaded onto a strong cation exchange spin column (MonoSpin SCX; GL Science, Tokyo, Japan). The peptide sample was washed twice with buffer B, eluted with 4%  $\text{NH}_4\text{OH}$  in MeOH, and evaporated to dryness. Samples were reconstituted in 50 mM ammonium bicarbonate and analyzed for total peptide concentration using BCA protein assay kit (ThermoFisher Pierce, Rockford, IL).

### 2.3. Detection and identification of peptides released from retinal pericytes

Reduction of 2  $\mu$ g of pericyte derived peptide samples was performed with 10 mM dithiothreitol for 1 h. The samples were then alkylated with 40 mM iodoacetamide in the dark for 1 h. The reaction was halted by the addition of 40 mM dithiothreitol for an additional 1 h. The reductive alkylation peptides were purified with a MonoSpin C18 spin column (GL Sciences) to remove any unreacted compounds. All reductive alkylation procedures were performed at room temperature. Nano LC-MS/MS was performed using a Paradigm MS4 system (Michrom BioResources, Auburn, CA) coupled to a QSTAR Elite Q-TOF mass spectrometer (Sciex, Framingham, MA) as described previously with minor modification at the nano liquid chromatography step (9). Peptides were eluted with mobile phases of A (2% acetonitrile, 0.1% formic acid) and B (90% acetonitrile, 0.1% formic acid) as follows: 0-30% B for 120 min, 30-40% B for 10 min, and 40-95% B for 15 min at a flow rate of 300 nL/min. Peptide identifications were acquired by matching raw spectra data against human protein from the International Protein Index (IPI) database using ProteinPilot software (version 3.0, Sciex) with the Paragon algorithm.

### 2.4. Synthesis of peptides

Peptides (ADT; SLPEAGPGRTLVSCKPQAHGAPAP PSGSAPHFL and  $\Delta$ ADT; LVSSCKPQAHGAPAP PSGSAPH) were synthesized by Biosynthesis (Lewisville, TX) and were obtained at > 98% purity. The peptide purity was verified by analytical reverse phase high performance liquid chromatography (HPLC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Peptides were solubilized in endotoxin-free water at a concentration of 10 mM and correctly diluted when used in the assays.

### 2.5. Endothelial cell proliferation assay

HRMVECs were seeded at a density of  $1 \times 10^4$  cells/well in a 24 well plate. After 24 h incubation, different concentrations of  $\Delta$ ADT and ADT (10 nM, 100 nM, and 1  $\mu$ M) were added to the wells and incubated for an additional 20 h. The cells were then trypsinized and counted against a control well.

### 2.6. Endothelial migration assay

For the endothelial migration assay using wound healing, transendothelial electrical resistance was measured by an electric cell-substrate impedance sensing (ECIS) Z $\theta$  instrument (Applied Biophysics, Troy, NY) as described previously (9). Briefly, HRMVECs were plated at confluence in 8W1E gold

electrode culture plates (Applied Biophysics) precoated with type I collagen in EGM-2 MV medium. On the following day, the medium was changed to serum starvation medium (EBM-2 with 0.5% fetal bovine serum), and the cells were incubated for an additional 16 h. The peptides were added to a final concentration of 1  $\mu$ M. After 1 h treatment, cells were submitted to an elevated voltage pulse frequency of 60 kHz, 1,400  $\mu$ A amplitude, and 20 s duration. This led to the death and detachment of cells present on the small active electrode, resulting in a wound that was normally healed by cells surrounding the small active electrode that had not been submitted to the elevated voltage pulse. Wound healing was then assessed by continuous impedance measurements at 16 kHz for 12 h.

### 2.7. *In vitro* endothelial tube formation assay

An endothelial tube formation assay used Matrigel (BD Biosciences, Bedford, MA) according to the manufacturer's protocol. Matrigel was thawed and 250  $\mu$ L aliquots were transferred into an eight well chamber slide and incubated at 37°C for 30 min. HRMVECs were seeded in quadruplicate ( $1 \times 10^5$  cells/well) in the presence of 100 nM  $\Delta$ ADT and incubated for 16 h at 37°C. At the end of the incubation period, cells were stained with calcein AM (BD Biosciences) and imaged by the IN Cell Analyzer 6000 (GE Healthcare Biosciences, Buckinghamshire, UK). The branch point and total length of tubes for each condition were measured using the IN Cell Analyzer.

### 2.8. Immunoblot analysis

Serum-starved HRMVECs were exposed for 5-120 min to 100 nM  $\Delta$ ADT. SDS-PAGE was performed by using standard procedures on 7.5% tris-glycine gels under reducing conditions. Proteins were transferred to Immobilon-P (Merck Millipore, Bedford, MA). The blots were incubated with rabbit anti-human focal adhesion kinase (FAK) antibody (1:1,000; Cell Signaling Technology, Beverly, MA) or rabbit anti-human phospho-FAK pTyr397 antibody (1:1,000; Cell Systems Technology). The immunoreactive protein was visualized using a SuperSignal West Femto reagent (ThermoFisher Pierce).

### 2.9. Filamentous actin staining

HRMVECs were plated on a type I collagen-coated eight well chamber slide (BD Biosciences) at  $1 \times 10^4$  cells/well in EGM-2 MV. On the following day, the media was replaced with serum-free basal media (EBM-2, 2% BSA). After 6 h incubation, 100 nM  $\Delta$ ADT was added to the wells and incubated for an additional 5, 30, or 90 min. Subsequently, cells were washed twice in PBS, fixed in 0.4 mL of 4% paraformaldehyde at

room temperature for 20 min, and washed with PBS. The cells were blocked and permeabilized with PBS containing 1% normal horse serum and 0.4% Triton X-100 overnight at 4°C. Cells were subsequently washed and incubated with Alexa Fluor 488 phalloidin (Molecular Probes, Carlsbad, CA) and 4',6-diamidino-2-phenylindole (DAPI; Dojindo, Kumamoto, Japan) in the aforementioned blocking buffer for 1 h at room temperature. The slides were washed twice in PBS, mounted on a microscope slide and FluoroSave reagent (Merk Millipore), and observed under a fluorescence microscope.

## 3. Results and Discussion

### 3.1. LC-MS/MS analysis of retinal pericytes derived from secreted peptides

To identify the secreted peptides from retinal pericytes, the peptide fractions were prepared from the culture fluid using column chromatography. We performed shotgun peptidomics experiments to investigate the bioactive peptides in the conditioned media of the cells. The LC-MS/MS data were submitted through the ProteinPilot software and searched against the human database with a decoy database to apply a false discovery rate. Using this technique, the 256 peptides originating from 114 genes could be identified with a confidence level of at least 95%. The 44 peptides originating from 15 genes that were identified at least twice in three independent experiments are listed in Table 1 (each peptide shows the results of the highest peptide score). The 42 peptides were identified with a confidence level of 99%, except for two peptides of thymosin  $\beta$ -10 with a confidence level of 97%. Interestingly, we identified Ala65-Ala96 and Ala65-Glu103 of reticulon-4, which play a role in inhibition of neurite outgrowth and cell spread (10). This finding indicates that since retinal pericytes are located outside retinal capillaries, they may interact with optic nerve cells. However, these 44 peptides included a fragment of cytoskeleton proteins, such as actin and vimentin. We selected five genes encoding an extracellular protein categorized as "secreted protein" in the UniProt database, namely ADM, annexin A1, annexin A2, interstitial collagenase, and vasorin.

The identified peptide of interstitial collagenase was only pro-peptide domain (11). Vasorin is predominantly expressed in vascular smooth muscle cells, and its expression is developmentally regulated. Vasorin is a transforming growth factor  $\beta$ -binding protein and has been shown to modulate the activity of growth factors (12). However, the peptide of vasorin identified in the present study was in the membrane spanning region. Annexin A1 is a member of the annexin family of calcium-lipid binding proteins, which are structurally defined by a highly conserved protein core domain

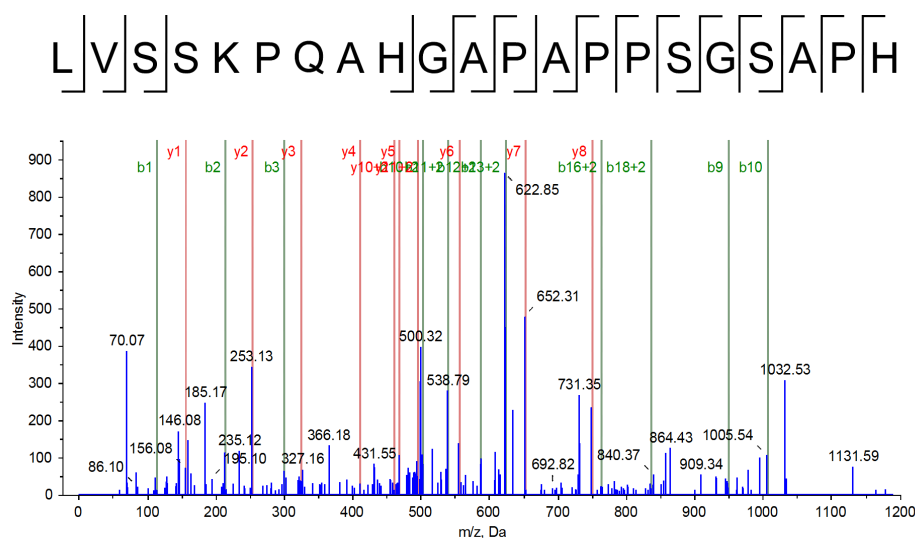


harboring calcium ions, phospholipid binding sites, and an N-terminal region that is unique for a given annexin (13). The anti-inflammatory activities elicited by exogenously applied annexin A1 are mediated through its unique N-terminal domain. Moreover, the identified peptide of annexin A1 was the N-terminal annexin 1 peptide, which is able to activate the human N-formyl peptide receptor (FPR) family members, FPR, FPR-like 1, or FPR-like 2 (14). All identified peptides (9-22 residues) of annexin A2 were in the N-terminal regions, for which physiological conditions have never before been reported. The N-terminal domain regulates the properties of the AnxA2 core by binding to S100A10 (15). The ADM gene encodes for a prohormone of 185 amino acid residues, which is post-translationally modified to generate four peptides: proadrenomedullin N-terminal 20 peptide (Ala22-Arg41), mid-regional pro-adrenomedullin (Glu45-Val92), adrenomedullin (Tyr95-Tyr146), and adrenotensin (ADT) (Ser153-Leu185) (16). In a search for novel regulators of retinal microvascular endothelial cells, we focused on a peptide derived from the ADT region of ADM gene products, because ADT has an endothelium-dependent vasoconstriction effect (17), and antagonizes the stimulatory effect of adrenomedullin during the generation of endothelial nitric oxide (18). Moreover, its function remains largely unknown. In particular, the identified peptide was a partial fragment of ADT lacking ten residues of the N-terminus and two residues of the C-terminus. The truncation mechanism of ADT is unknown; however, detection of this truncated peptide was reproducible in independent experiments. Therefore, we named the peptide  $\Delta$ ADT. A representative  $\Delta$ ADT peptide identified by LC-MS/MS is shown in Figure 1.

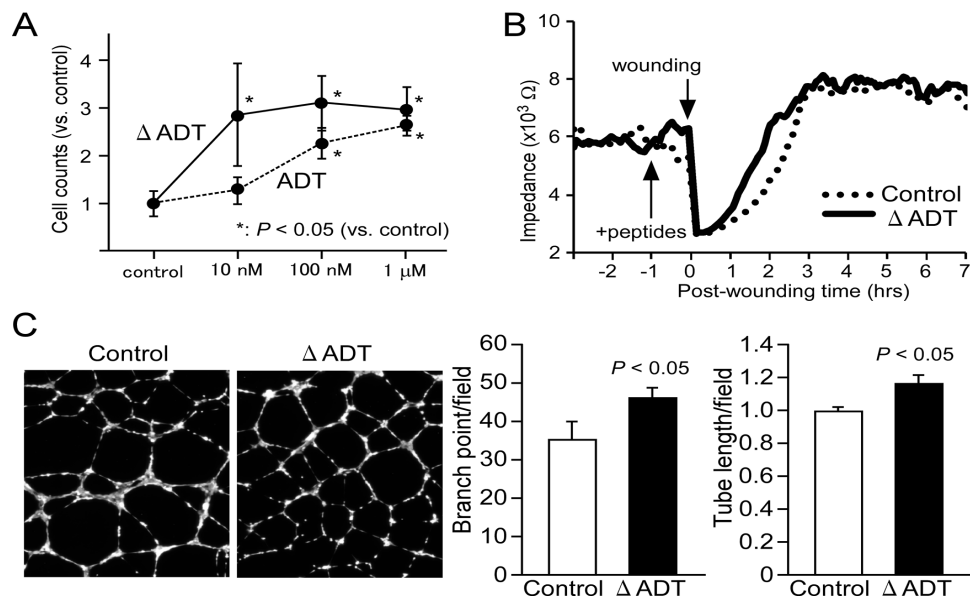
### 3.2. $\Delta$ ADT stimulates angiogenesis in cultured HRMVEC

We investigated whether  $\Delta$ ADT could activate the proliferation of HRMVECs compared with ADT and control vehicle (water). To investigate the effect of  $\Delta$ ADT on HRMVECs, we assayed proliferation by cell counting. Figure 2A shows that  $\Delta$ ADT increases the proliferation of HRMVEC in a dose-dependent manner. Moreover, the cells that were treated with the  $\Delta$ ADT showed increased cell growth compared with ADT treated cells. The maximal effect of  $\Delta$ ADT was significantly lower at 100 nM than that of ADT at a concentration of 1  $\mu$ M ( $p < 0.05$ ). This result indicates that ADT and  $\Delta$ ADT are able to stimulate the proliferation of endothelial cells, and suggests that  $\Delta$ ADT has a higher proliferation rate than ADT. In addition, this finding is consistent with reports showing the effects of ADT on upregulated proliferation in rat mesangial cells and pulmonary arterial smooth muscle cells (19,20).

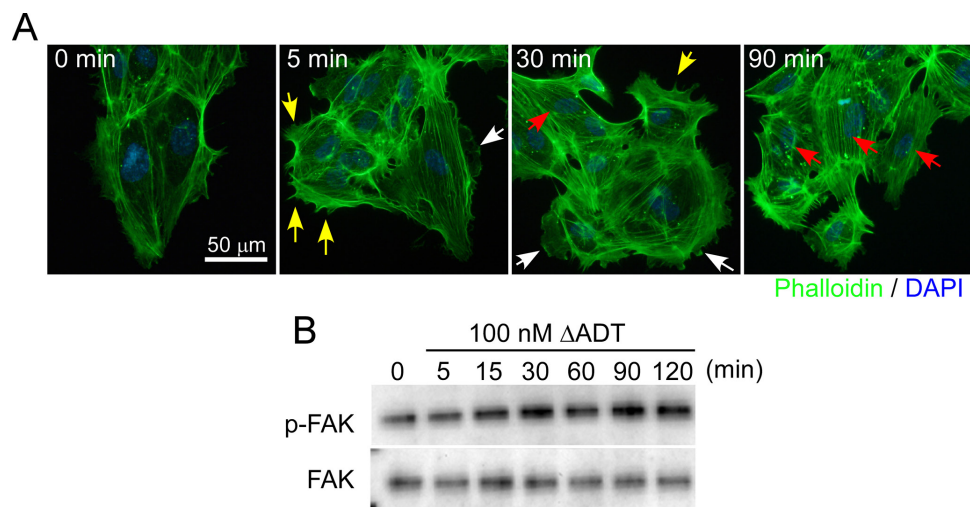
Next, we performed HRMVEC migration assay by early wound healing using ECIS. As shown in Figure 2B,  $\Delta$ ADT-treated cells exhibited nearly complete healing of the wound after 2 h, although control vehicle-treated cells remained in half recovery. This result indicates that  $\Delta$ ADT increases endothelial cell migration. To evaluate the effect of  $\Delta$ ADT on the ability of endothelial cells to form tube-like structures, an *in vitro* tube formation assay was performed. HRMVECs treated with  $\Delta$ ADT increased compared with control vehicle angiogenic tube formation, as quantitatively evaluated by branch point numbers and total tube length (Figure 2C). Considered together, our data showed that  $\Delta$ ADT derived from retinal pericytes is a novel angiogenic peptide in HRMVECs.



**Figure 1.** Typical tandem mass spectrometry (MS/MS) spectrum for the identification of a truncated peptide of adrenotensin (Leu163-His183) ( $\Delta$ ADT) in culture fluid of human retinal pericytes. The MS/MS spectrum of precursor ion  $\Delta$ ADT (LVSSKPKQAHGAPAPPSSGSAPH) with an identified  $m/z$  of 499.02 ( $z = 4$ ).



**Figure 2. Promoting effect of a truncated peptide of adrenotensin ( $\Delta$ ADT) on proliferation, migration, and tube formation of human retinal microvascular endothelial cells (HRMVECs). (A)** Cell proliferation assay of HRMVEC incubated with  $\Delta$ ADT (solid line), ADT (dashed line), and control vehicle (water) for 20 h. Cell number was determined by direct counting of trypsinized cell suspensions. **(B)** Cell migration dynamics during wound-healing assay. After 1 h treated with 1  $\mu$ M  $\Delta$ ADT (solid line) or control vehicle (dotted line), HRMVECs were submitted to electrical wounding. Post-wound migration of cells was measured by real-time monitoring using electric cell-substrate impedance sensing (ECIS). **(C)**  $\Delta$ ADT increases *in vitro* tube formation in HRMVECs compared with the control vehicle. Fluorescence imaging, counting of branch point number ( $n = 4$ ;  $p < 0.05$ ), and measurement of tube length ( $n = 4$ ;  $p < 0.05$ ) were conducted by IN Cell Analyzer.



**Figure 3.  $\Delta$ ADT induced actin reorganization and phosphorylation of focal adhesion kinase (FAK) in HRMVEC. (A)** After treatment with 100 nM truncated peptide of adrenotensin ( $\Delta$ ADT) for 0, 5, 30, and 90 min, human retinal microvascular endothelial cells (HRMVECs) were representative cells at filamentous actin and nuclei were stained with Alexa Fluor 488 phalloidin and 4',6-diamidino-2-phenylindole (DAPI), respectively. Yellow arrow; filopodia, white arrow; membrane ruffling, red arrow; stress fiber. **(B)**  $\Delta$ ADT induces FAK phosphorylation on Tyr397 in a time-dependent manner. Representative images of western blots are shown.

To determine whether the  $\Delta$ ADT signaling pathway plays a role in regulating the polarization of the actin cytoskeleton, we evaluated the immunocytochemical location of actin in HRMVEC treated with or without  $\Delta$ ADT. Figure 3A shows representative images of the reorganization of the actin cytoskeleton induced by 100 nM  $\Delta$ ADT. In unstimulated serum-starved cells, actin filaments were localized mainly in the cortical region of the cells (0 min). After 5 min of stimulation with 100 nM  $\Delta$ ADT, the cells

formed filopodia and membrane ruffling. Following prolonged treatment, the cells displayed prominent stress fibers related to cell contraction across the cell body (30 and 90 min). Focal adhesion kinase (FAK) is the pivotal molecule that controls the formation of focal adhesions, thereby providing the platform for cells to generate the locomotive force. Endothelial FAK is essential for vascular morphogenesis and vascular repair due to its central role in endothelial cell migration (21,22). To clarify whether FAK is

activated through autophosphorylation at Tyr397 by  $\Delta$ ADT stimulation, we performed an immunoblot analysis with the HRMVECs after  $\Delta$ ADT treatment. As shown in Figure 3B, tyrosine phosphorylation of FAK was increased in a time-dependent manner, and the levels of phosphorylation peaked at 30 min. This finding suggests that  $\Delta$ ADT activates tyrosine phosphorylation of endothelial FAK, and implies that this leads to the formation of focal adhesion complexes. These results indicate that  $\Delta$ ADT induces alterations in actin cytoskeleton dynamics and focal adhesion in endothelial cells.

In the present study, we identified  $\Delta$ ADT derived from human retinal pericytes by LC-MS/MS analysis, and suggest that  $\Delta$ ADT is a novel angiogenic peptide in endothelial cells.  $\Delta$ ADT may play a role in angiogenesis as a novel factor of pericyte-endothelial cell interactions. At present, ADT and  $\Delta$ ADT receptors remain unknown, but future research should provide further elucidation of these molecules. Within the context of retinal disease therapy, the above findings suggest that administering  $\Delta$ ADT receptor antagonist or using antibodies against  $\Delta$ ADT peptide may be effective for the treatment of retinal angiogenesis.

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### References

- Orlidge A, D'Amore PA. Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. *J Cell Biol.* 1987; 105:1455-1462.
- Balabanov R, Dore-Duffy P. Role of the CNS microvascular pericyte in the blood-brain barrier. *J Neurosci Res.* 1998; 53:637-644.
- Dohgu S, Takata F, Yamauchi A, Nakagawa S, Egawa T, Naito M, Tsuruo T, Sawada Y, Niwa M, Kataoka Y. Brain pericytes contribute to the induction and up-regulation of blood-brain barrier functions through transforming growth factor- $\beta$  production. *Brain Res.* 2005; 1038:208-215.
- Hori S, Ohtsuki S, Hosoya K, Nakashima E, Terasaki T. A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation *in vitro*. *J Neurochem.* 2004; 89:503-513.
- Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res.* 2005; 97:512-523.
- Clynen E, Baggerman G, Veelaert D, Cerstiaens A, Van der Horst D, Harthoorn L, Derua R, Waelkens E, De Loof A, Schoofs L. Peptidomics of the pars intercerebralis-corpora cardiaca complex of the migratory locust, *Locusta migratoria*. *Eur J Biochem.* 2001; 268:1929-1939.
- Schrader M, Schulz-Knappe P. Peptidomics technologies for human body fluids. *Trends Biotechnol.* 2001; 19:S55-60.
- Sasaki K, Satomi Y, Takao T, Minamino N. Snapshot peptidomics of the regulated secretory pathway. *Mol Cell Proteomics.* 2009; 8:1638-1647.
- Takahashi E, Okumura A, Unoki-Kubota H, Hirano H, Kasuga M, Kaburagi Y. Differential proteome analysis of serum proteins associated with the development of type 2 diabetes mellitus in the KK-A<sup>y</sup> mouse model using the iTRAQ technique. *J Proteomics.* 2013; 84:40-51.
- Oertle T, van der Haar ME, Bandtlow CE, Robeva A, Burfeind P, Buss A, Huber AB, Simonen M, Schnell L, Brosamle C, Kaupmann K, Vallon R, Schwab ME. Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. *J Neurosci.* 2003; 23:5393-5406.
- Clark IM, Cawston TE. Fragments of human fibroblast collagenase. Purification and characterization. *Biochem J.* 1989; 263:201-206.
- Ikeda Y, Imai Y, Kumagai H, Nosaka T, Morikawa Y, Hisaoka T, Manabe I, Maemura K, Nakaoka T, Imamura T, Miyazono K, Komuro I, Nagai R, Kitamura T. Vasorin, a transforming growth factor  $\beta$ -binding protein expressed in vascular smooth muscle cells, modulates the arterial response to injury *in vivo*. *Proc Natl Acad Sci U S A.* 2004; 101:10732-10737.
- Gerke V, Moss SE. Annexins: From structure to function. *Physiol Rev.* 2002; 82:331-371.
- Ernst S, Lange C, Wilbers A, Goebeler V, Gerke V, Rescher U. An annexin I N-terminal peptide activates leukocytes by triggering different members of the formyl peptide receptor family. *J Immunol.* 2004; 172:7669-7676.
- Rety S, Sopkova J, Renouard M, Osterloh D, Gerke V, Tabaries S, Russo-Marie F, Lewit-Bentley A. The crystal structure of a complex of p11 with the annexin II N-terminal peptide. *Nat Struct Biol.* 1999; 6:89-95.
- Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. *Endocr Rev.* 2000; 21:138-167.
- Gumusel B, Chang JK, Hyman A, Lippman H. Adrenotensin: An ADM gene product with the opposite effects of ADM. *Life Sci.* 1995; 57:PL87-90.
- Li J, Ren Y, Dong X, Zhong G, Wu S, Tang C. Roles of different peptide fragments derived from proadrenomedullin in the regulation of vascular tone in isolated rat aorta. *Peptides.* 2003; 24:563-568.
- Xue H, Yuan P, Zhou L, Yao T, Huang Y, Lu LM. Effect of adrenotensin on cell proliferation is mediated by angiotensin II in cultured rat mesangial cells. *Acta Pharmacol Sin.* 2009; 30:1132-1137.
- Li W, Kong QY, Zhao CF, Zhao F, Li FH, Xia W, Wang R, Hu YM, Hua M. Adrenomedullin and adrenotensin regulate collagen synthesis and proliferation in pulmonary arterial smooth muscle cells. *Braz J Med Biol Res.* 2013; 46:1047-1055.
- Braren R, Hu H, Kim YH, Beggs HE, Reichardt LF, Wang R. Endothelial FAK is essential for vascular network stability, cell survival, and lamellipodial formation. *J Cell Biol.* 2006; 172:151-162.
- Chien S, Li S, Shiu YT, Li YS. Molecular basis of mechanical modulation of endothelial cell migration. *Front Biosci.* 2005; 10:1985-2000.

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