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Loss of smooth muscle cell disintegrin and metalloproteinase 17 transiently suppresses angiotensin II-induced hypertension and end-organ damage

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ABSTRACT

Hypertension is associated with hypertrophy and hyperplasia of smooth muscle cells (SMCs). Disintegrin and metalloproteinase 17 (ADAM17) is a membrane-bound enzyme reported to mediate SMC hypertrophy through activation of epidermal growth factor receptor (EGFR). We investigated the role of ADAM17 in Ang II-induced hypertension and end-organ damage. VSMC was isolated from mice with intact ADAM17 expression (*Adam17*$^{f/f}$) or lacking ADAM17 in the SMC (*Adam17*$^{f/f}$/*Cre*^{Sm22}). Human VSMCs were isolated from the aorta of donors, and *ADAM17* deletion was achieved by siRNA transfection. Ang II suppressed proliferation and migration of *Adam17*-deficient SMCs, but did not affect apoptosis (mouse and human), this was associated with reduced activation of EGFR and Erk1/2 signaling. *Adam17*$^{f/f}$/*Cre*^{Sm22} and littermate *Adam17*$^{f/f}$ mice received saline or Ang II (Alzet pumps, 1.5 mg/kg/d; 2 or 4 weeks). Daily blood pressure measurement in conscious mice (telemetry) showed suppressed hypertension in *Adam17*$^{f/f}$/*Cre*^{Sm22} mice during the first week of Ang II infusion, but by the second week, it become comparable to that in *Adam17*$^{f/f}$ mice. EGFR activation remained suppressed in *Adam17*$^{f/f}$/*Cre*^{Sm22}-Ang II arteries. *Ex vivo* vascular function and compliance assessed in mesenteric arteries were comparable between genotypes. Consistent with the transient protection against Ang II-induced hypertension, *Adam17*$^{f/f}$/*Cre*^{Sm22} mice exhibited significantly lower cardiac hypertrophy and fibrosis, and renal fibrosis at 2 weeks post-Ang II, however this protection was abolished by the fourth week of Ang II infusion. In conclusion, while *Adam17*-deficiency suppresses Ang II-induced SMC remodeling *in vitro*, *in vivo Adam17*-deficiency provides only a transient protective effect against Ang II-mediated hypertension and end-organ damage.

1. Introduction

Hypertension is a significant health problem that continues to underlie the morbidity and mortality associated with cardiovascular diseases [1]. Angiotensin II is the central product of the renin-angiotensin system, and is a key player in the etiology of hypertension and associated pathologies such as cardiovascular and renal diseases. Vascular remodeling, mainly hypertrophy and hyperplasia of vascular smooth muscle cells (VSMC), are hallmarks of hypertension. Ang II is a strong hypertensive agent that, in addition to triggering SMC contraction [2], promotes VSMC proliferation, hypertrophy and migration; thereby contributing to vascular remodeling [3]. The main pathway identified for this function of Ang II has been reported to be through activation of the angiotensin II receptor type-1 (AT_1) and transactivation of epidermal growth factor receptor (EGFR) [4,5]. EGFR can be activated by a number of ligands including heparin-binding epidermal growth factor-like growth factor (HB-EGF), transforming growth factor-alpha (TGFα), amphiregulin, β-cellulin, epigen, and epiregulin [6]. A disintegrin and metalloprotease 17 (ADAM17) is a membrane-bound enzyme that can proteolytically cleave and release a number of membrane-bound growth factors and cytokines [7], and thereby it can regulate multiple cell functions. A role for ADAM17 has been reported in cultured VSMCs [8], and its loss was recently linked to short-term protection against Ang II-induced cardiac hypertrophy [9].

In this study, we aimed to determine the long-term impact of ADAM17 loss in SMCs in response to Ang II-mediated hypertension and end-organ damage, and to identify the underlying mechanism. We used *in vitro* culture systems of mouse and human VSMCs, and mice with SMC-specific *Adam17* deletion (*Adam17*$^{flox/flox}$/*Sm22αCre*,

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Adam17$^{f/f}$/*Cre*^{Sm22}) to demonstrate that loss of *Adam17* in SMCs results in a transient suppression of Ang II-induced hypertension, hypertrophy and fibrosis, however this protective effect dissipated with prolonged Ang II infusion.

2. Materials and methods

The detailed Materials and methods section is available in the Supplementary File.

2.1. Experimental animals

Mice lacking *Adam17* in SMCs (*Adam17*$^{flox/flox}$/*Sm22αCre*, *Adam17*$^{f/f}$/*Cre*^{Sm22}) were generated by cross-breeding mice carrying floxed *Adam17* alleles (*Adam17*$^{tm1.2Bbl}$/J, *Adam17*$^{f/f}$) with mice expressing Cre-recombinase under the control of the smooth muscle cell promoter (*Sm22α-Tg*(*Tagln-cre*)*1Her*/J, Cre^{Sm22}). Litter-mate male *Adam17*$^{f/f}$ and *Adam17*$^{f/f}$/*Cre*^{Sm22} mice were used in this study. All experiments were conducted in accordance with the guidelines of the University of Alberta Animal Care and Use Committee (ACUC) and the Canadian Council of Animal Care.

2.2. Angiotensin II pump implantation

Alzet micro-osmotic pumps were implanted subcutaneously in 10 to 12 weeks old mice of either genotype as previously described [10].

2.3. Telemetric blood pressure measurement

The effect of SMC *Adam17* deletion on Ang II-induced blood pressure changes in conscious and active mice of either genotype was evaluated using telemetry as described [11].

2.4. Ex vivo pressure myography

After 2 weeks of saline or Ang II infusion, second-order mesenteric arteries from either genotype were used for *ex vivo* pressure myography assessment as previously described [12].

2.5. Primary mouse and human vascular smooth muscle cell isolation and culture

Primary human and mouse VSMCs were isolated using a modified enzyme dispersal method [13,14]. Human and mouse VSMCs were used at passages 3–5 for experiments to minimize phenotypic switching which can occur with prolonged culture period and at later passages. Cells were serum-deprived for 24 h prior to the start of the experimental protocols.

2.6. Human ADAM17 siRNA transfection

ADAM17-knockdown in human VSMCs *in vitro* was achieved by *ADAM17*-siRNA oligonucleotide pairs as previously described [15].

2.7. BrdU incorporation assay

Human VSMCs transfected with scrambled siRNA or human *ADAM17*-siRNA pairs, and mouse VSMCs from *Adam17*$^{f/f}$ or *Adam17*$^{f/f}$/*Cre*^{Sm22} were incubated with 10 μmol/L BrdU (5-bromo-2′-deoxyuridine, Abcam)-containing serum free culture medium in the presence or absence of Ang II (1 μmol/L) for 24 h. After being stained with FITC-conjugated anti-BrdU antibody, the rate of VSMC proliferation was quantified by the number of BrdU-positive cells as a percentage of total number of cells (DAPI-positive) in the same field.

2.8. Wound healing assay

The effect of SMC *Aadam17* deletion on Ang II-induced human and mouse VSMC migration was assessed using an *in vitro* wound healing assay as described previously [16].

2.9. Flow cytometric analysis

Cell apoptosis was evaluated by using an Annexin V-PE/7-AAD staining kit (BD Biosciences) and a BD LSR Fortessa-SORP cytometer (BD Biosciences). Data were analyzed using the FlowJo software (version 10.0.7, TreeStar, Inc.).

2.10. RNA expression analysis

Messenger RNA expression levels for *Adams-10*, *−12*, *−15*, *−17*, and *−19*, Ang II receptor type 1 (*At1*), and Ang II receptor type 2 (*At2*) in mouse VSMCs after 24 h of vehicle (saline) or Ang II (1 μmol/L) treatment were measured by TaqMan RT-PCR as previously described [15]. The expression levels of aforementioned ADAMs were also measured in the saline and Ang II (2 weeks and 4 weeks) infused mesenteric arteries from both *Adam17*$^{f/f}$ and *Adam17*$^{f/f}$/*Cre*^{Sm22} mice. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was used as an internal control.

2.11. Immunohistological analyses

Saline or Ang II infused hearts and kidneys were paraffin-embedded and processed for Masson's trichrome and picrosirius red (PSR) staining as previously described [15,17]. Collagen content was quantified from PSR-stained sections using Image Pro Plus software (version 6.0, Media Cybernetics). Briefly, 8 high power fields were randomly selected from each heart and kidney section (total $n = 80$ images per group/genotype) for quantification. The collagen content in each examined filed was calculated as PSR positive-to-total tissue area ratio. The value for *Adam17*$^{f/f}$-saline group was set at 1.

2.12. Statistics

Averaged data are presented as mean ± SEM. For comparison of the main effects of 2 factors (*Adam17*-deficiency and Ang II treatment) 2-way ANOVA followed by Bonferroni's *post-hoc* tests were used. Statistics were carried out using the IBM SPSS statistics 21 software, and bar graphs were plotted using the OriginPro 2015 software. Statistically significance was recognized at $p < 0.05$.

3. Results

3.1. Loss of ADAM17 does not alter the general morphology of mouse or human VSMCs

Purity of vascular smooth muscle cell (VSMC) cultures was >95% (mouse and human) as indicated by immunofluorescence staining for SMC-specific proteins SM22α, SM-MHC11 (smooth muscle myosin heavy chain 11), and calponin (Fig. 1Ai and Bi). VSMCs isolated from *Adam17*$^{f/f}$/*Cre*^{Sm22} mice showed a complete loss of ADAM17 (Fig. 1Aii). We used two human *ADAM17* siRNAs to knock-down *Adam17* expression in these cells, both of which resulted in >80% reduction in ADAM17 protein with siRNA #2 (s13720) showing a higher efficacy (Fig. 1Bii). Loss of ADAM17 did not alter the appearance of the VSMCs (Fig. 1Ai and Bi), nor the expression levels of contractile proteins (Fig. 1Aii and Bii). However, we found that after passage 7, expression of these VSMC proteins markedly decreased in both groups (data not shown). As such, all of the *in vitro* experiments in this study were performed at passages 3–5.