ACTA ABP BIOCHIMICA POLONICA

Vol. 68, No 4/2021
745–750
https://doi.org/10.18388/abp.2020_5645

Regular paper

mRNA Expression of thrombospondin 1, 2 and 3 from proximal to distal in human abdominal aortic aneurysm – preliminary report

Aleksandra Augusciak-Duma[1]✉, Marta Lesiak[1], Karolina L. Stepien[1], Ewa Gutmajster[2] and Aleksander L. Sieron[1]

[1]Department of Molecular Biology, Faculty of Medical Science in Katowice, Medical University of Silesia, Katowice, Poland; [2]Department of Medical Genetics, Faculty of Medical Science in Katowice, Medical University of Silesia, Katowice, Poland

Abdominal aortic aneurysm is a process involving the disruption and reconstruction of the extracellular matrix and the apoptosis of smooth muscle cells under the strong influence of the immune system. Thrombospondins are proteins that influence a wide range of cell-matrix interactions. While *THBS1* and *THBS2* are widely studied, the effects of *THBS3* on extracellular matrix and vascular cells are poorly understood. Additionally, it is not known whether expression of these genes' changes along the aneurysm tissue. Here we analyzed the expression of THBSs mRNA isolated from the harvested tissues along the aneurysm divided into three zones based on their morphology. Total mRNA was isolated from 13 male patients undergoing scheduled open aortic repair, with each aneurysm divided into a proximal part, an aneurysm bag, and a distal part with border tissue as a control. Two step real-time PCR analysis with random hexamers was performed, which allowed the detection of significantly increased expression of all analyzed thrombospondins, especially *THBS3*, at the control tissue. Overexpression of THBSs may have a destabilizing effect on the structure of the extracellular matrix by affecting both the matrix producing cells and by inhibiting the activity of matrix proteins.

Keywords: abdominal aortic aneurysm; pathology; thrombospondins; gene expression; THBS3

Received: 29 March, 2021; revised: 20 May, 2021; accepted: 14 June, 2021; available on-line: 20 October, 2021

✉e-mail: aaugusciak@sum.edu.pl

Acknowledgements of Financial Support: The work was in part financially supported by the Institutional grant KNW-1-049/N/9/0 (awarded to AAD). The equipment for molecular analyses used in this work was purchased from the Silesian Bio-Farma Center for Biotechnology, Bioengineering and Bioinformatics Project (no POIG.02.01.00-00-166/08 THE OPERATIONAL PROGRAMME INNOVATIVE ECONOMY FOR 2007-2013. Priority Axis 2).

Abbreviations: AAA, abdominal aortic aneurysm; ECM, extracellular matrix; MMP, matrix metalloproteinase; THBS, thrombospondin; VEGF, vascular endothelial growth factor

INTRODUCTION

Abdominal aortic aneurysm (AAA) is a heterogeneous asymptomatic disease which, if not detected, can lead to death from aortic rupture (Keisler & Carter, 2015; Sakalihasan *et al.*, 2005). Aortic dilation results from persistent inflammation (Liu *et al.*, 2015a; MA_3RS Study Investigators 2017; Sawyer *et al.*, 2016), associated with death of endothelial (EC) and smooth muscle cells (SMC) (Kokot *et al.*, 2013; Siasos *et al.*, 2015) and abnormal remodeling of the extracellular matrix (ECM) (Quintana & Taylor, 2019; Didangelos *et al.*, 2011; Kadoglou & Liapis, 2004). An increased risk of developing AAA is strongly correlated with gender, age, smoking, family history of AAA, atherosclerotic diseases, spinal cord injury, and genetic predispositions (Sakalihasan *et al.*, 2005; Lederle *et al.*, 2000; Li *et al.*, 2013). In the overall European population, the prevalence in 2011–2013 was 4.3%, with 80% mortality resulting from AAA rupture (Li *et al.*, 2013). In the Polish population aged over 65 years, the incidence of AAA is 2.62% and almost 4 times higher in men (4.32%) than in women (1.23%) (Mikołajczyk-Stecyna *et al.*, 2013; Tkaczyk *et al.*, 2019).

Thrombospondins (THBSs) seem to be important inhibitors of AAA, keeping the growth of the aneurysm under control. Thrombospondins are classified in the family of secreted glycoproteins that have very complex and often opposite biological functions. They contain domains for interacting with cell surfaces, growth factors, cytokines, and ECM components (Adams & Lawler, 2011). Trimeric A subfamily consists of *THBS1* and *THBS*2 and has been studied in AAA, pentameric *THBS3* is less studied, and its function and importance in angiogenesis and vascularization are poorly understood (Stenina-Adognravi, 2013).

Thrombospondin 1 was shown to be involved in the maintenance of vascular structure by affecting cell proliferation, apoptosis, and adhesion (Liu *et al.*, 2015b). Numerous studies revealed the involvement of *THBS1* in the development of AAA through acceleration of vascular inflammation, activation of *TGF-beta,* and activation of the cofilin pathway (Jana *et al.*, 2020; Liu *et al.*, 2015b; Krishna *et al.*, 2015; Adams & Lawler, 2011; Resovi *et al.*, 2014; Crawford *et al.*, 1998; Yamashiro *et al.*, 2018). *THBS1* is also one of the proteins involved in the "angiogenic switch" that changes the phenotype of endothelial cells from quiescent to sprouting (Lawler & Lawler, 2011). *THBS2* cooperates with *THBS1* for most of its functions (Lawler & Lawler, 2011). *THBS2* plays an important role in the structural and functional heart integrity (Golledge *et al.*, 2013a). *THBS3* promotes sarcolemmal destabilization by reducing integrin function (Schips *et al.*, 2019). Overexpression of *THBS3* in the heart of mice uniformly inhibits the expression of integrins α4, 5, 6, 7, 8, 9, 10, and β1D, which leads to the rupture of the cell membrane. The lack of *THBS3* expression protects the heart from pressure overload (Schips *et al.*, 2019).

In presented study, we aimed at detection of the expression pattern of thrombospondins along the AAA using the border segment of aneurysm as a control (Ziaja,

2013; Legaki *et al*., 2020). Bearing in mind that AAA is a multifactorial and heterogeneous disease with great variability between patients, it is difficult to select the ideal control group to compare expression levels. Therefore, the best way to achieve it was to compare the affected tissue with the phenotypically healthy tissue excised next to the abdominal aortic aneurysm.

MATERIALS AND METHODS

Patient characteristics

A total of 13 samples from males were collected following AAA surgery from patients who were scheduled for open aortic repair (OAR). The patients who underwent surgery for AAA included in this study were male and ranged in age from 57 to 82 years old (mean 67.15±6.47 years) (Supplementary Table I at https://ojs.ptbioch.edu.pl/index.php/abp/). The AAA patients excluded from the study were those who fulfilled the following criteria: (a) chronic obstructive pulmonary disease (COPD); (b) diabetes; (c) creatinine level >1.0; (d) reconstruction of coronary vessels and thoracic aorta (CABG); (e) reconstruction of carotid artery (ICA); (f) diagnosed generalized atherosclerosis (AO); (g) family history of AAA or inherited cardiovascular syndromes; and (h) lack of ability to provide informed consent for surgical treatment.

The study plan was approved by the Bioethics Committee of the Medical University of Silesia in Katowice, no. KNW/0022/KB1/55/14 issued on June 17th, 2014, and its further extension no. KNW/0022/KB1/55/1/14/17 issued on June 27th, 2017.

Materials

Fragments of AAA, on average 50 mm in diameter, were collected from the patients upon surgery. When technically feasible, non-aneurysmal aortic samples of the aneurysm neck (unaffected samples, as confirmed by pathologists) were simultaneously collected and used as controls (Fig. 1).

All surgical procedures were performed in the planned mode. Briefly, the material collected for the research was part of the aneurysm excised during an OAR (ICD9 - 38.424). The samples were collected intra-surgically at the General and Vascular Surgery Department in Katowice-Ochojec, Poland, and secured immediately in the surgery room, at the room temperature and placed in sterile 50 mL tubes filled with 25 mL of high glucose (4.5 mg/mL) Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) supplemented with penicillin (10000 U/ml), streptomycin (10 mg/ml), and amphotericin B (25 μg/ml) (PAA Laboratories, Pasching, Austria). The described procedures ensured the maintenance of alive cells, thus, prevent RNA degradation. Then, upon arrival at the cell culture facility, aneurysmal tissue was divided into 4 fragments: border and control/border (C); neck – upper/proximal (1); aneurysm bag or middle/central (2); and the end segment – bottom/distal region (3), where the second part was the aneurysm sack of the excised AAA (Ziaja, 2013). The most altered portion of tissue was called the "aneurysm sac," and the surrounding tissue were marked as proximal and distal parts. The method of tissue fragmentation was based on the research of Ziaja (Ziaja, 2013). The control tissue was marked as control by the surgeon performing the surgery, from this part, histological examinations were performed to confirm removal of the aneurysm in its entirety by the method of margin analysis (Legaki, 2020). From the fragments, specimens of ~4 mm × 4 mm × 2–4 mm were immediately subjected to RNA isolation and purification (Fig. 1).

Methods

Total RNA was isolated in duplicate using Zymogen Quick RNA Mini Prep (Ambion, Austin, Texas, USA) following sample homogenization in TissueLyser II (Qiagen, Venlo, The Netherlands). Quality and quantity evaluation was performed using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). Total RNA (1 to 2 μg) was transcribed using a cDNA Transcriptor First Strand cDNA Synthesis Kit (Roche, Penzberg, Upper Bavaria, Germany) with random hexamers. Expression analyses with Real Time Custom Panel 384-96 (config. no 100131839; Roche) and LightCycler 480 Probe Master (Roche, Penzberg, Upper Bavaria, Germany) were performed using a LightCycler 480 II (Roche, Penzberg, Upper Bavaria, Germany). The genes analyzed in this report are listed in Table 1.

Gene expression profiling

Gene expression was analyzed using GenEx ver6 software (MultiD Analyses AB; Sweden). Analyses were performed using the $Ct^{(2\text{-}\Delta\Delta Ct)}$ comparative method as follows: raw data were normalized to sample amount fol-

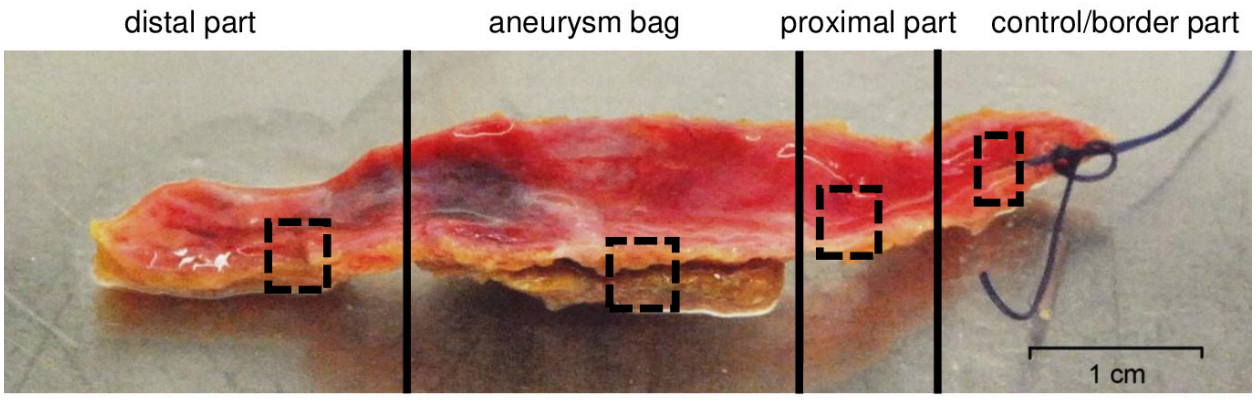

Figure 1. Abdominal aortic aneurysm collected during an open aortic repair (OAR) schematically showing the plan of partitioning of the tissue.
From each segment, pieces of ~4 mm x 4 mm x 2–4 mm were subjected immediately to RNA isolation and purification (boxed).

Table 1. Alphabetical list of genes used in the study with appropriate Assay IDs (Roche) and HGNC symbols.

Assay ID	Gene Symbol	Description
141139	GAPDH	glyceraldehyde-3-phosphate dehydrogenase [HGNC:4141; Gene ID:2597]; Forward primer sequence: AGCCACATCGCTCAGACAC; Reverse primer sequence: GCCCAATACGACCAAATCC; UPL Probe number: 60
144221	GUSB	glucuronidase, beta [HGNC:4696; Gene ID:2990]; Forward primer sequence: TCGCCATCAACAACACACTC; Reverse primer sequence: TCTGGACAAAGTAACCCTTGG; UPL Probe number: 77
102088	PPIA	peptidylprolyl isomerase A (cyclophilin A) [HGNC:9253; Gene ID:5478]; Forward primer sequence: TTCATCTGCACTGCCAAGAC; Reverse primer sequence: CACTTTGCCAAACACCACAT; UPL Probe number: 158
102119	RPL13A	small nucleolar RNA, C/D box 32A [HGNC:10159; Gene ID:26819]; Forward primer sequence: CTGGACCGTCTCAAGGTGTT; Reverse primer sequence: GCCCCAGATAGGCAAACTT; UPL Probe number: 157
104740	THBS1	thrombospondin 1 [HGNC:11785; Gene ID:7057]; Forward primer sequence: GCTGCACTGAGTGTCACTGTC; Reverse primer sequence: TCAGGAACTGTGGCATTGG; UPL Probe number: 43
104742	THBS2	thrombospondin 2 [HGNC:11786; Gene ID:7058]; Forward primer sequence: AGCGTCAGATGTGCAACAAG; Reverse primer sequence: GGAAGCAGGGGTTGGATAA; UPL Probe number: 158
112464	THBS3	thrombospondin 3 [HGNC:11787; Gene ID:7059]; Forward primer sequence: TGAGCAATCCTACCCAGACA; Reverse primer sequence: TTGTCCTTGGTGTCCTGATG; UPL Probe number: 112

lowed by normalization to the reference genes *GAPDH*, *GusB*, *PPIA*, and *RPL13a* (Table 1). Relative expression of target genes was calculated with the comparison against the control/border samples.

Statistical analyses

The Kolmogorov–Smirnov test was employed to determine if the data from the expression analysis showed a normal distribution. As the data were not normally distributed, a nonparametric test (Mann-Whitney 1-tailed test) was used for analysis of the data (Weissgerber *et al.*, 2015). The threshold for the *p*-value was set to less than 0.05. For the determination of the differential expression of genes, scatter plot analysis was used with a significance area equal to 1. Spearman correlation coefficients (r_S) were calculated to determine the correlation between genes.

RESULTS AND DISCUSSION

The expression of all analyzed genes in majority samples from all sections of the aneurysm as well as from the border section were detected at mRNA level. Only one proximal sample revealed negative results (Supplementary Table II at https://ojs.ptbioch.edu.pl/index.php/abp/).

To the best of our knowledge, this is the first report on *THBS1*, *THBS2,* and *THBS3* expression in human samples along the aneurysm tissue and its border at the mRNA level. On the basis of the relative expression of the samples, we observed that the expression of thrombospondins mRNA is different in the same patient in all four sections of the aneurysm analyzed, but unfortunately too few samples (7 patients with all sections) did not allow statistical results to be obtained, and so we could only observe the trends (Supplementary Fig. I at https://ojs.ptbioch.edu.pl/index.php/abp/).

When combined, significant differences between expression of the three thrombospondins in different parts of surgically removed aneurysm were found (Fig. 2, Table 2). Previous reports from animal models revealed that *THBS1* overexpression in a mouse model of AAA largely contributed to its development by inhibiting the expression of *TIMP1* (tissue inhibitor of metalloproteinase 1), which allows inflammatory macrophages to infiltrate vascular tissues. Moreover, mice without *THBS1*

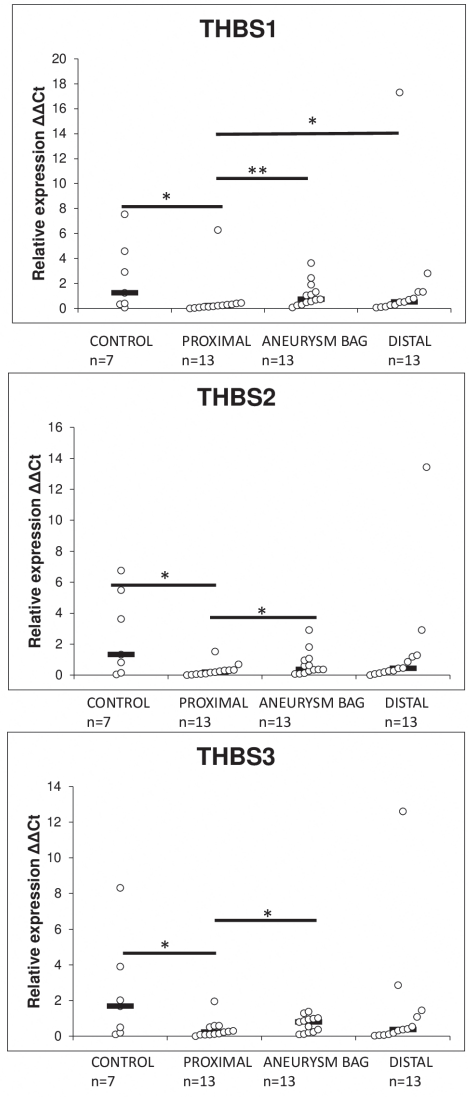

Figure 2. Relative expression of thrombospondins.
The Y axis represents the relative expression Nrel=2^(ΔCnormalizedtarget-ΔCcontrol), and the X axis represents the expression of genes in the control, proximal, aneurysm sack and distal segments. *Mann-Whitney test, 1-tailed $p<0.05$; **Mann-Whitney test, 1-tailed $p<0.005$

Table 2. Fold change of expression between segments of the AAA from the results of scatter plot evaluation.

Gene	Fold change (FD – fold decrease; FI – fold increase)					
	Proximal part *versus* border tissue	Aneurysm bag	Distal part	Aneurysm bag *vs.* proximal part	Distal part *vs.* proximal part	Distal part *vs.* Aneurysm bag
THBS1	↓ 3.4* FD	↓ 2.5 FD	1.3 FD	1.4** FI	↑ 2.5* FI	1.9 FD
THBS2	↓ 4.8* FD	↓ 3.6 FD	1.9 FD	1.3* FI	↑ 2.5 FI	1.9 FI
THBS3	↓ 4.0* FD	↓ 3.3 FD	1.8 FD	1.2* FI	↑ 2.2 FI	1.8 FD

↓decreased expression according to the scatter plot correlation; ↑increased expression according to the scatter plot correlation; *Mann-Whitney test, 1-tailed $p<0.05$; **Mann-Whitney test, 1-tailed $p<0.005$

did not reduce the levels of *Mmp9*, *Mmp3*, *Mmp10*, and *Mmp12* (Yang *et al.*, 2020). In our work, the highest expression of *THBS1* was found in the border tissue and the difference between the border part and the next proximal part was significant. There was also a significant increase in mRNA expression in the aneurysm sac compared with the proximal segment. The same relation was detected between the proximal and distal segments. However, *THBS1* expression in the affected segments did not reach levels of that found in control tissues (Supplementary Fig. II at https://ojs.ptbioch.edu.pl/index.php/abp/). High levels of THBS expression in the control tissue could mark an extensive inflammation in these tissues, which may contribute to AAA development.

In the mouse model, 70% of *THBS1* was expressed by macrophages, which mainly invade the adventitious layer (composed of endothelial cells) (Liu *et al.*, 2015a; Yang *et al.*, 2020). Therefore, the conclusion could be made that in an already developed aneurysm, the highest macrophage infiltration occurs not in the tissue already phenotypically changed but in the tissue that is still considered as healthy.

The highest change fold ratio between border tissue and the proximal part was for the *THBS2* (4.89±0.003) (Table 2). The expression pattern was similar to that of *THBS1*. This result was not surprising as in numerous studies these proteins showed a similar function under physiological and pathological conditions (Lawler & Lawler; Zhang *et al.*, 2009; Colombo *et al.*, 2010; Yamauchi *et al.*, 2007; Oganesian *et al.*, 2008; Dawson *et al.*, 1997; Jiménez *et al.*, 2000; Sun *et al.*, 2009). Additionally, in the correlation analysis using the Spearman correlation coefficient, strong positive correlations between *THBS2* and *THBS1* (rS=0.76 ±0) and between *THBS2* and *THBS3* (rS=0.74 ±0) were found. The analysis in silico of gene expression omnibus set (GEO) (Wan *et al.*, 2018) revealed enrichment of differentially expressed genes (DEG) for *THBS2* in human abdominal aneurysm, but not as a potential biomarker or candidate gene for drug therapy. In 2007 only dataset addressing AAA – GDS2838 (http://www.ncbi.nlm.nih. gov/geo/) was published, but based on the information of its profiles for *THBS1*, *THBS2* and *THBS3* there is no final conclusion as to theirs rank, they are too heterogenous. Most researchers either take a sample of the AAA center or extract mRNA from the entire aneurysm and compare it with samples taken from a "healthy" aorta during another procedure or after death. We propose a different approach, similar to that used in the analysis of cancer tissues. Thus, the affected tissues are similarly divided with the marginal/border tissue treated as a control sample, thus eliminating individual differences between samples collected from different individuals and focusing only on the differences between different sections of the aneurysm. As shown in the results, these differences are universal and are not due to individual differences.

Recently, first reports of increased expression levels of *THBS2* have been described in protein studies in human AAA tissue samples, (Qi *et al.*, 2020). Elevated *THBS2* concentration in serum plasma was associated with the risk of cardiac mortality in patients with AAA (Golledge *et al.*, 2013b) and aortic dissection (AD) (Qi *et al.*, 2020). Furthermore, its polymorphism was linked to hypertension susceptibility (Yamada, 2009). *THBS2* protein was detected in smooth muscle cells and at a lower level in endothelial cells where it positively correlated with *TNF-a* and *IL-6* suggesting that *THBS2* may regulate the inflammatory response (Qi *et al.*, 2020). *THBS2* also reduced the presence of active *MMP2*. *THBS2*-deficient mouse fibroblasts have been reported to produce pro-*MMP2* twice compared with wild-type cells (Yang *et al.*, 2000).

Here, too, we present the analysis of *THBS3* expression in human AAA for the first time. There was a significant expression decrease between the border tissue and the proximal part of the aneurysm (change fold=4.01±0.02) and between the proximal part and the aneurysm sac (change fold=1.2±0.02). Additionally, a strong positive correlation of expression was found between *THBS3* and *THBS1* (r_S=0.76 ±0), and between *THBS3* and *THBS2* (r_S=0.74 ±0). *THBS3* is the least known member of the thrombospondin family. Particularly, its function in AAA is poorly understood. In an aging mouse model *THBS3* expression was increased in the myocardial ECM of elder mice, which may lead to *Smad2* activation in epithelial cells and age-related cardiac inflammation (Toba *et al.*, 2016). In contrast, the expression level of *THBS3* was not associated with pulmonary arterial hypertension (PAH), although *THBS1* was directly involved in the activation of TGF-β in the mouse PAH model and was both required and sufficient for the development of PAH (Kumar *et al.*, 2017). In osteosarcomas, *THBS3* expression is correlated as a prognostic factor of worse overall survival and as a stimulator of tumor progression due to its high ability to promote angiogenesis (Dalla-Torre *et al.*, 2006). Multi-omics analysis of vascular calcification also highlighted the unknown role of *THBS3* in this process, possibly by imposing sarcolemmal instability (Qian *et al.*, 2021; Schips *et al.*, 2019). Overexpression of *THBS3* is the result of a response to an inflammatory process. Improved remodeling, expansion, and fibrosis of the ventricles in experimental mice was a result of inhibition of integrin expression, which led to rupture of the cell membrane (Schips *et al.*, 2019).

Vascular smooth muscle cells, which are an essential component of the aorta (they constitute >90% cells in there) during AAA development, undergo excessive ap-

optosis followed by destruction of the dynamic balance of ECM (Quintana & Taylor, 2019). *THBS1* and *2* enhance apoptosis by promoting the expression of inflammatory factors and activating apoptotic pathways, as well as by inhibiting angiogenesis (Armstrong & Bornstein, 2003; Lawler, 2000). Since they play a protective role for healthy tissue under severe macrophage attack at the onset of inflammation in the aorta, thrombospondins respond by eliminating the potential source of inflammation. In chronic conditions, their overexpression leads to disruption of homeostasis in the ECM and then to a positive feedback loop when overexpression of MMPs and its inhibitors, remodel the matrix. In addition, the hemodynamic load pressure contributes to further imbalance, and with *THBS3* overexpression, it additionally disturbs the stability and the presence of integrins on the cell membrane.

CONCLUSION

We found that there was a significantly variable expression pattern in the aneurysm tissue taking into account the segmentation of the AAA. The most prominent feature found was the sudden increase in expression in the marginal tissue compared to the aneurysm segments. Based on our and previous studies, we hypothesize that *THBS3* downregulates the expression of integrins, which can affect cell membranes and destabilize the complex junction of the ECM structure in the aortic vessel. The destabilization of the ECM and the connection of cells to it may lead to ease the access of *THBS1*-expressing macrophages to the ECM of the abdominal aorta. The further overexpression of *THBS1* might induce structural changes in the ECM by, for example, inhibition of the *TIMP1* at protein level, while overexpression of *THBS2* in the endothelial and smooth muscle cells inhibits the *MMP2* protein further destabilizing the correct balance in ECM homeostasis.

Our research has its limitations. First, the sample size in the experiment was small and a larger number of tissue samples are needed to confirm our findings. Second, mRNA expression is not a precise indicator of the level of protein in tissues and its specific location. More research is needed to understand the molecular mechanisms by which thrombospondins work in AAA.

Acknowledgements

The authors would like to acknowledge Prof. Krzysztof Ziaja and Prof. Wacław Kuczmik from the Department of General and Vascular Surgery, Medical University of Silesia, Katowice, Poland, for constructive medical consultation of the manuscript and granting the tissues of AAA.

REFERENCES

Adams JC, Lawler J (2011) The Thrombospondins. *Cold Spring Harb. Perspect. Biol.* **3**: a009712–a009712. https://doi.org/10.1101/cshperspect.a009712

Armstrong LC, Bornstein P (2003) Thrombospondins 1 and 2 function as inhibitors of angiogenesis. *Matrix Biol.* **22**: 63–71. https://doi.org/10.1016/S0945-053X(03)00005-2

Colombo G, Margosio B, Ragona L, Neves M, Bonifacio S, Annis DS, Stravalaci M, Tomaselli S, Giavazzi R, Rusnati M, Presta M, Zetta L, Mosher DF, Ribatti D, Gobbi M, Taraboletti G (2010) Non-peptidic thrombospondin-1 mimics as fibroblast growth factor-2 inhibitors: An integrated strategy for the development of new antiangiogenic compounds. *J. Biol. Chem.* **285**: 8733–8742. https://doi.org/10.1074/jbc.M109.085605

Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SMF, Lawler J, Hynes RO, Boivin GP, Bouck N (1998) Thrombospondin-1 is a major activator of TGF-β1 *in vivo*. *Cell* **93**: 1159–1170. https://doi.org/10.1016/S0092-8674(00)81460-9

Dalla-Torre CA, Yoshimoto M, Lee C-H, Joshua AM, Rc De Toledo S, Petrilli AS, Andrade JA, Chilton-Macneill S, Zielenska M, Squire JA (2006) Effects of THBS3, SPARC and SPP1 expression on biological behavior and survival in patients with osteosarcoma. *BMC Cancer* **6**: 237. https://doi.org/10.1186/1471-2407-6-237

Dawson DW, Pearce SFA, Zhong R, Silverstein RL, Frazier WA, Bouck NP (1997) CD36 mediates the *in vitro* inhibitory effects of thrombospondin-1 on endothelial cells. *J. Cell Biol.* **138**: 707–717. https://doi.org/10.1083/jcb.138.3.707

Didangelos A, Yin X, Mandal K, Saje A, Smith A, Xu Q, Jahangiri M, Mayr M (2011) Extracellular matrix composition and remodeling in human abdominal aortic aneurysms: A proteomics approach. *Mol. Cell. Proteomics* **10**: https://doi.org/10.1074/MCP.M111.008128

Golledge J, Clancy P, Hankey GJ, Norman PE (2013a) Relation between serum thrombospondin-2 and cardiovascular mortality in older men screened for abdominal aortic aneurysm. *Am. J. Cardiol.* **111**: 1800–1804. https://doi.org/10.1016/J.AMJCARD.2013.02.038

Golledge J, Clancy P, Hankey GJ, Norman PE (2013b) Relation between serum thrombospondin-2 and cardiovascular mortality in older men screened for abdominal aortic aneurysm. *Am. J. Cardiol.* **111**: 1800–1804. https://doi.org/10.1016/j.amjcard.2013.02.038

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7084

Jana S, Chute M, Hu M, Winkelaar G, Owen CA, Oudit GY, Kassiri Z (2020) ADAM (a Disintegrin and Metalloproteinase) 15 deficiency exacerbates Ang II (Angiotensin II)–induced aortic remodeling leading to abdominal aortic aneurysm. *Arterioscler. Thromb. Vasc. Biol.* **40**: 1918–1934

Jiménez B, Volpert O V., Crawford SE, Febbraio M, Silverstein RL, Bouck N (2000) Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat. Med.* **6**: 41–48. https://doi.org/10.1038/71517

Kadoglou NP, Liapis CD (2004) Matrix metalloproteinases: contribution to pathogenesis, diagnosis, surveillance and treatment of abdominal aortic aneurysms. *Curr. Med. Res. Opin.* **20**: 419–432. https://doi.org/10.1185/030079904125003143

Keisler B, Carter C (2015) Abdominal Aortic Aneurysm. *Am. Fam. Physician*. **91**: 538–543

Kokot M, Biolik G, Ziaja D, Fojt T, Cisak K, Antoniak K, Pawlicki K, Ziaja K, Dualwa J (2013) Endothelium injury and inflammatory state during abdominal aortic aneurysm surgery: Scrutinizing the very early and minute injurious effects using endothelial markers – A pilot study. *Arch. Med. Sci.* **9**: 479–486. https://doi.org/10.5114/aoms.2013.34412

Krishna SM, Seto SW, Jose RJ, Biros E, Moran CS, Wang Y, Clancy P, Golledge J (2015) A peptide antagonist of thrombospondin-1 promotes abdominal aortic aneurysm progression in the angiotensin II-infused apolipoprotein-E-deficient mouse. *Arterioscler. Thromb. Vasc. Biol.* **35**: 389–398. https://doi.org/10.1161/ATVBAHA.114.304732

Kumar R, Mickael C, Kassa B, Gebreab L, Robinson JC, Koyanagi DE, Sanders L, Barthel L, Meadows C, Fox D, Irwin D, Li M, McKeon BA, Riddle S, Dale Brown R, Morgan LE, Evans CM, Hernandez-Saavedra D, Bandeira A, Maloney JP, Bull TM, Janssen WJ, Stenmark KR, Tuder RM, Graham BB (2017) TGF-β activation by bone marrow-derived thrombospondin-1 causes Schistosoma- and hypoxia-induced pulmonary hypertension. *Nat. Commun.* **8**: 1–13. https://doi.org/10.1038/ncomms15494

Lawler J (2000) The functions of thrombospondin-1 and -2. *Curr. Opin. Cell Biol.* **12**: 634–640. https://doi.org/10.1016/S0955-0674(00)00143-5

Lawler PR, Lawler J (2012) Molecular Basis for the Regulation of Angiogenesis by Thrombospondin-1 and-2. *Cold Spring Harb. Perspect. Med.* **2**: a006627. https://doi.org/10.1101/cshperspect.a006627

Lederle FA, Johnson GR, Wilson SE, Chute EP, Hye RJ, Makaroun MS, Barone GW, Bandyk D, Moneta GL, Makhoul RG (2000) The aneurysm detection and management study screening program. *Arch. Intern. Med.* **160**: 1425. https://doi.org/10.1001/archinte.160.10.1425

Legaki E, Siasos G, Klonaris C, Athanasiadis D, Patelis N, Sioziou A, Oikonomou E, Liakakos T, Gazouli M, Tousoulis D (2020) Mir-335-5p as a potential regulator of LRP1 expression in abdominal aortic aneurysm. *Hell. J. Cardiol.* **61**: 430–432. https://doi.org/10.1016/j.hjc.2020.01.002

Li X, Zhao G, Zhang J, Duan Z, Xin S (2013) Prevalence and trends of the abdominal aortic aneurysms epidemic in general population--a meta-analysis. *PLoS One* **8**: e81260. https://doi.org/10.1371/journal.pone.0081260

Liu Z, Morgan S, Ren J, Wang Q, Annis DS, Mosher DF, Zhang J, Sorenson CM, Sheibani N, Liu B (2015a) Thrombospondin-1 (TSP1) contributes to the development of vascular inflammation by regulating monocytic cell motility in mouse models of abdominal aortic aneurysm. *Circ. Res.* **117**: 129–141. https://doi.org/10.1161/CIRCRESAHA.117.305262

Liu Z, Morgan S, Ren J, Wang Q, Annis DS, Mosher DF, Zhang J, Sorenson CM, Sheibani N, Liu B (2015b) Thrombospondin-1 (TSP1) contributes to the development of vascular inflammation by regulating monocytic cell motility in mouse models of abdominal aortic aneurysm HHS public access. *Circ Res* **117**: 129–141. https://doi.org/10.1161/CIRCRESAHA.117.305262

MA_3RS Study Investigators (2017) Aortic wall inflammation predicts abdominal aortic aneurysm expansion, rupture, and need for surgical repair. *Circulation* **136**: 787–797. https://doi.org/10.1161/CIRCULATIONAHA.117.028433

Mikołajczyk-Stecyna J, Korcz A, Gabriel M, Pawlaczyk K, Oszkinis G, Omski RS (2013) Risk factors in abdominal aortic aneurysm and in Polish population aortoiliac occlusive disease and differences between them. *Sci. Rep.* **3**: 3528. https://doi.org/10.1038/srep03528

Oganesian A, Armstrong LC, Migliorini MM, Strickland DK, Bornstein P (2008) Thrombospondins use the VLDL receptor and a nonapoptotic pathway to inhibit cell division in microvascular endothelial cells. *Mol. Biol. Cell* **19**: 563–571. https://doi.org/10.1091/mbc.E07-07-0649

Plana E, Gálvez L, Medina P, Navarro S, Fornés-Ferrer V, Panadero J, Miralles M (2020) Identification of novel microRNA profiles dysregulated in plasma and tissue of abdominal aortic aneurysm patients. *Int. J. Mol. Sci.* **21**: 4600. https://doi.org/10.3390/ijms21134600

Qi L, Wu K, Shi S, Ji Q, Miao H, Bin Q (2020) Thrombospondin-2 is upregulated in patients with aortic dissection and enhances angiotensin II-induced smooth muscle cell apoptosis. *Exp. Ther. Med.* **20**: 150. https://doi.org/10.3892/etm.2020.9279

Qian Y, Li L, Sun Z, Liu J, Yuan W, Wang Z (2021) A multi-omics view of the complex mechanism of vascular calcification. *Biomed. Pharmacother.* **135**: 111192. https://doi.org/10.1016/j.biopha.2020.111192

Quintana RA, Taylor WR (2019) Cellular mechanisms of aortic aneurysm formation. *Circ. Res.* **124**: 607–618. https://doi.org/10.1161/CIRCRESAHA.118.313187

Resovi A, Pinessi D, Chiorino G, Taraboletti G (2014) Current understanding of the thrombospondin-1 interactome. *Matrix Biol.* **37**: 83–91. https://doi.org/10.1016/j.matbio.2014.01.012

Sakalihasan N, Limet R, Defawe O (2005) Abdominal aortic aneurysm. *Lancet* **365**: 1577–1589. https://doi.org/10.1016/S0140-6736(05)66459-8

Sawyer DM, Pace LA, Pascale CL, Kutchin AC, O'Neill BE, Starke RM, Dumont AS (2016) Lymphocytes influence intracranial aneurysm formation and rupture: role of extracellular matrix remodeling and phenotypic modulation of vascular smooth muscle cells. *J. Neuroinflammation* **13**: 185. https://doi.org/10.1186/s12974-016-0654-z

Schips TG, Vanhoutte D, Vo A, Correll RN, Brody MJ, Khalil H, Karch J, Tjondrokoesoemo A, Sargent MA, Maillet M, Ross RS, Molkentin JD (2019) Thrombospondin-3 augments injury-induced cardiomyopathy by intracellular integrin inhibition and sarcolemmal instability. *Nat. Commun.* **10**: 76. https://doi.org/10.1038/s41467-018-08026-8

Siasos G, Mourouzis K, Oikonomou E, Tsalamandris S, Tsigkou V, Vlasis K, Vavuranakis M, Zografos T, Dimitropoulos S, Papaioannou T, Kalampogias A, Stefanadis C, Papavassiliou A, Tousoulis D (2015) The Role of Endothelial Dysfunction in Aortic Aneurysms. *Curr. Pharm. Des.* **21**: 4016–4034. https://doi.org/10.2174/1381612821666150826094156

Stenina-Adognravi O (2013) Thrombospondins. *Curr. Opin. Lipidol.* **24**: 401–409. https://doi.org/10.1097/MOL.0b013e3283642912

Sun J, Hopkins BD, Tsujikawa K, Perruzzi C, Adini I, Swerlick R, Bornstein P, Lawler J, Benjamin LE (2009) Thrombospondin-1 modulates VEGF-A-mediated Akt signaling and capillary survival in the developing retina. *Am. J. Physiol. Circ. Physiol.* **296**: H1344–H1351. https://doi.org/10.1152/ajpheart.01246.2008

Tkaczyk J, Przywara S, Terpiłowski M, Brożyna K, Ilżecki M, Terlecki P, Zubilewicz T (2019) Prevalence and risk factors of abdominal aortic aneurysm among over 65 years old population in Lublin, Poland. *Acta Angiol.* **25**: 1–6. https://doi.org/10.5603/AA.2019.0001

Toba H, de Castro Brás LE, Baicu CF, Zile MR, Lindsey ML, Bradshaw AD (2016) Increased ADAMTS1 mediates SPARC-dependent collagen deposition in the aging myocardium. *Am. J. Physiol. - Endocrinol. Metab.* **310**: E1027–E1035. https://doi.org/10.1152/ajpendo.00040.2016

Wan L, Huang J, Ni H, Yu G (2018) Screening key genes for abdominal aortic aneurysm based on gene expression omnibus dataset. *BMC Cardiovasc. Disord.* **18**: https://doi.org/10.1186/s12872-018-0766-8

Weissgerber TL, Milic NM, Winham SJ, Garovic VD (2015) Beyond bar and line graphs: Time for a new data presentation paradigm. *PLOS Biol.* **13**: e1002128. https://doi.org/10.1371/journal.pbio.1002128

Yamada (2009) Association of polymorphisms of THBS2 and HSPA8 with hypertension in Japanese individuals with chronic kidney disease. *Mol. Med. Rep.* **2**: 205-211. https://doi.org/10.3892/mmr_00000085

Yamashiro Y, Thang BQ, Shin SJ, Lino CA, Nakamura T, Kim J, Sugiyama K, Tokunaga C, Sakamoto H, Osaka M, Davis EC, Wagenseil JE, Hiramatsu Y, Yanagisawa H, Yoshito Yamashiro, Bui Quoc Thang, Seung Jae Shin, Caroline Antunes Lino, Tomoyuki Nakamura, Jungsil Kim, Kaori Sugiyama, Chiho Tokunaga HS, Motoo Osaka, Elaine C. Davis, Jessica E. Wagenseil, Yuji Hiramatsu HY (2018) Role of thrombospondin-1 in mechanotransduction and development of thoracic aortic aneurysm in mouse and humans. *Circ. Res.* **123**: 660–672. https://doi.org/10.1161/CIRCRESAHA.118.313105

Yamauchi M, Imajoh-Ohmi S, Shibuya M (2007) Novel antiangiogenic pathway of thrombospondin-1 mediated by suppression of the cell cycle. *Cancer Sci.* **98**: 1491–1497. https://doi.org/10.1111/j.1349-7006.2007.00534.x

Yang H, Zhou T, Sorenson CM, Sheibani N, Liu B (2020) Myeloid-derived TSP1 (Thrombospondin-1) contributes to abdominal aortic aneurysm through suppressing tissue inhibitor of metalloproteinases-1. *Arterioscler. Thromb. Vasc. Biol.* **40**: E350–E366. https://doi.org/10.1161/ATVBAHA.120.314913

Yang Z, Kyriakides TR, Bornstein P (2000) Matricellular proteins as modulators of cell-matrix interactions: Adhesive defect in thrombospondin 2-null fibroblasts is a consequence of increased levels of matrix metalloproteinase-2. *Mol. Biol. Cell* **11**: 3353–3364. https://doi.org/10.1091/mbc.11.10.3353

Zhang X, Kazerounian S, Duquette M, Perruzzi C, Nagy JA, Dvorak HF, Parangi S, Lawler J (2009) Thrombospondin-1 modulates vascular endothelial growth factor activity at the receptor level. *FASEB J.* **23**: 3368–3376. https://doi.org/10.1096/fj.09-131649

Ziaja D (2013) *The patency in inferior artery, detachment of thrombus, extensiveness of aneurysm, expression of cytokines and proteines compared to content of metals and non-metals as a marker of inflamation of the infrarenal aortic aneurysm*. 1st edn. Katowice: Medical University of Silesia, Poland