Human pulmonary artery endothelial cells in the model of mucopolysaccharidosis VI present a prohypertensive phenotype

Adam Golda a,⁎, Agnieszka Jurecka b,⁎⁎, Karolina Gajda c, Anna Tylki-Szymańska b, Anna Lalik c

a Department of Cardiology, Glwice Medical Center, Gliwice, Poland
b Department of Pediatrics, Nutrition and Metabolic Diseases, The Children’s Memorial Health Institute, Warsaw, Poland
c Systems Engineering Group, Faculty of Automatic Control, Electronics and Informatics, Silesian University of Technology, Gliwice, Poland

A R T I C L E   I N F O
Article history:
Received 13 October 2014
Received in revised form 20 February 2015
Accepted 21 February 2015
Available online 28 February 2015

Keywords:
Pulmonary hypertension
Endothelial cells
Proliferation
Apoptosis
Endothelial nitric oxide synthase
Natriuretic peptide type C
Vascular endothelial growth factor

A B S T R A C T
Background: Mucopolysaccharidosis type VI (MPS VI) is an autosomal recessive lysosomal disorder caused by a deficient activity of N-acetylgalactosamine-4-sulfatase (ARSB). Pulmonary hypertension (PH) occurs in MPS VI patients and is a marker of bad prognosis. Malfunction of endothelium, which regulates vascular tonus and stimulates angiogenesis, can contribute to the occurrence of PH in MPS VI.

Aim: The aim of the study was to establish a human MPS VI cellular model of pulmonary artery endothelial cells (HPAECs) and evaluate how it affects factors that may trigger PH such as proliferation, apoptosis, expression of endothelial nitric oxide synthase (eNOS), natriuretic peptide type C (NPPC), and vascular endothelial growth factor A (VEGFA).

Results: Increasing concentrations of dermatan sulfate (DS) reduce the viability of the cells in both ARSB deﬁciency and controls, but hardly influence apoptosis. The expression of eNOS in HPAECs is reduced up to two thirds in the presence of DS. NPPC shows a biphasic expression reaction with an increase at 50 μg/mL DS and reduction at 0 and 100 μg/mL DS. The expression of VEGFA decreases with increasing DS concentrations and absence of elastin, and increases with increasing DS in the presence of elastin.

Conclusion: Our data suggest that MPS VI endothelium presents a prohypertensive phenotype due to the reduction of endothelium’s proliferation ability and expression of vasorelaxing factors.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Mucopolysaccharidosis type VI (MPS VI, Maroteaux–Lamy syndrome, OMIM# 253200) is an autosomal recessive lysosomal storage disorder affecting many organs of the human body. It is determined by mutations in the arylsulfatase B gene (ARSB) located on chromosome 5 (5q13–5q14) [1]. Pathogenic mutations of this gene result in a reduced or absent activity of arylsulfatase B (4-sulfatase, arylsulfatase B, ARSB, EC 3.1.6.12), and lead to incomplete degradation and accumulation of glycosaminoglycans (GAGs); dermatan sulfate (DS) and chondroitin 4-sulfate (CS). The negative role of DS on the cardiovascular system has been widely documented; the excess of DS in the heart causes valvular heart disease with all consequences [2,3]. The occurrence of pulmonary hypertension (PH) in MPS patients is a marker of increased mortality [4] whereas the etiology and mechanisms causing PH in MPS VI patients still remain unknown.

Pulmonary hypertension is caused by the reduction of pulmonary vessels. Reduced total cross-section of pulmonary vessels causes the given blood volume from the right ventricle to be ejected with higher pressure to retain the constant flow. Moreover, increased wall stiffness of pulmonary vessels contributes to increased pulmonary pressure as the vascular bed cannot receive blood volume quickly enough. There is a large heterogeneity among patients with PH in terms of disease development, including genetic and clinical predispositions and clinical responses to available therapeutics [5]. PH is characterized by vasoconstriction and vascular remodeling including medial thickness and muscularization of non-muscularized vessels [6–8]. Most patients with severe pulmonary arterial hypertension also have structural changes in pulmonary arterioles caused by the angiogenic proliferation of endothelial cells forming plexiform lesions [9–11]. Endothelium plays a key regulatory function in the vascular system by controlling vascular tone, platelet activity, leukocyte adhesion and angiogenesis [12]. For occurrence and progression of severe pulmonary hypertension the dysfunction of endothelium is crucial; it affects the reduction of vasodilator production, whereas vasoconstrictors are overexpressed [13,14]. Moreover, pulmonary artery endothelial cell apoptosis, induced by a
variety of factors (i.e. hypoxisa), is the initial step triggering PH [15–17]. Most endothelial functions depend, to various extent, on changes in intracellular calcium [18]. The endothelial nitric oxide synthase (eNOS) producing nitric oxide (NO) remains the main regulator causing vascular smooth muscle relaxation and blood pressure reduction. It is well documented that the increase of intracellular calcium stimulates (via calmodulin) the eNOS to produce NO [19]; on the other hand a calcium independent eNOS pathway has also been reported [20]. Another factor that proved to cause vasodilatation is the natriuretic peptide type C (NPPC) [21]. NPPC involves the calcium/nitric oxide synthase/nitrite oxide pathway for vascular relaxation [21]. Apart from vasodilatory properties, NPPC has anti-inflammatory [22–24] and anti-mitogenic properties [22,23,25] and promotes regeneration of endothelial cells in injured vessels [25–27]. Vascular endothelial growth factor (VEGF) is the main proangiogenic factor. VEGF’s under-expression is linked to PH occurrence [28,29]. It could be observed that in the monocrotaline PH model a drop of pulmonary expression of VEGF causes the decrease of pulmonary vessel number and increase in vessel wall thickness [29]. VEGF gene transfer in this model was an effective method of preventing the PH development and progression [30].

Cells interact with the extracellular matrix adhesively, however various signals can be transduced through the plasma membrane using cell surface receptors [31,32]. A non-integron receptor, which is present on the cell surface for interactions with elastin, consists of two cell–membrane-associated proteins immobilizing the third protein — elastin binding protein (EBP) [33–35]. This endothelial elastin–laminin receptor [36] is linked to cytoskeletal actin microfilaments and contains a lectin domain that, when occupied by galactosur or N-acetylgalactosamine-containing glycosaminoglycans (i.e. dermata sulfate), inhibits elastin binding [34,37,38]. The binding of elastin to the receptor causes a rapid transient intracellular calcium (Ca²⁺) rise and thus the intracellular calcium signaling contributes to the regulation of various endothelial functions [39]. Such a mechanism can at least partially explain the pathology of MPS VI on a cellular basis. Using this information we constructed MPS VI cellular model of human pulmonary artery endothelial cells where the arylsulfatase B was silenced with corresponding small interfering ribonucleic acid (siRNA) and the cells were cultured in the presence of dermata sulfate with or without elastin [40].

The aim of the study was to establish a human pulmonary artery endothelial model of MPS VI and evaluate how it alternates proliferation, apoptosis, expression levels of eNOS, NPPC and vascular endothelial growth factor A (VEGFA) — factors that can contribute to an elevated incidence of PH in MPS VI patients. New data obtained on endothelial cell dysfunction in MPS VI could contribute to the development of novel strategies for the treatment of this disorder.

2. Material and methods

Dermatan sulfate (chondroitin sulfate B sodium salt) from porcine intestinal mucosa and soluble elastin from bovine neck ligament obtained were from Sigma–Aldrich St. Louis, MO, USA.

Human pulmonary artery endothelial cells (HPAECs) were obtained from Lonza, Switzerland. EBM–2 Basal Medium supplied with EGM-2 BulletKit from Lonza, Switzerland was the growth medium. 13% Bovine serum albumin (BSA) from Lonza, Switzerland and EBM-2 Basal Medium supplied with EGM-2 BulletKit from Lonza, Switzerland was added to the growth medium to maintain the proliferation of the cells. The measurements were done in triplicates with control group transfected with non-targeting siRNA. The gene expression analyses were done 36 h after transfection.

To analyze the effect of DS and elastin on HPAECs the transfection medium was changed to growth medium after 24 hour transfection, than DS was added. After following 12 hour incubation elastin was added. The measurements were completed after the next 24 hour incubation.

RealTime PCR was done using RealTime PCR Mix EvaGreen Kit (A&A Biotechnology, Gdynia, Poland) and CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The expression levels of individual mRNA were analyzed using the cycle threshold method. Relative quantification of mRNA expression levels was calculated using the 2ΔΔCt method [41]. For RealTime PCR primers with the following sequences were used:

ARSB — Forward (F): ctcgctttttcacgtctcc, Reverse (R): cgcgctctctgttaacgcct.

VEGFA — F: ctcgcctcgttcgctcagg, R: cagggattgagagaaggtc.

eNOS — F: ttccaaactcgtcgtgaaa, R: gttacgctgcatcgcaga.

NPPC — F: gaaagggctccagatgcggct, R: gttcggcagtccagggc.

Relative quantification of cell viability was done using CellTiter 96® AQueous Non–Radioactive Cell Proliferation Assay (Promega, Fitchburg, USA) referred to as MTT Test. Shortly, the assay is based on the reduction of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. Only living and metabolic active cells can produce the formazan dye. The intensity of the product color measured at 490 nm is directly proportional to the number of living cells in the culture. The formazan dye product was quantified by a microplate spectrophotometer (EPOCH, BioTek Instruments, Winooski, VT, USA).

For quantification of apoptosis FITC Annexin V Apoptosis Detection Kit from BioLegend, San Diego, CA, USA was used. Shortly, the assay is based on the translocation of phosphatidylserine (PS) from the inner (cytoplasmic) leaflet of the plasma membrane to the outer (cell surface) leaflet in early stages of apoptosis. FITC–Annexin V binds PS and stained (apoptotic) cells are counted by flow cytometric analysis. To distinguish apoptotic (FITC–Annexin V staining) from necrotic cells FITC–Annexin V was used in conjunction with propidium iodide. Analysis of stained cells was performed using cytofluorometric FACSariaII (Becton Dickinson, Franklin Lakes, NJ, USA).

The statistical analyses were conducted using Microsoft Excel software (Microsoft Corporation, Redmond WA, USA). The value was expressed as mean ± standard deviation of three independent experiments. Two-tailed Student t-test was used for analyzing unpaired data, and p value below 0.05 was considered statistically significant.

3. Results

Using the commercially designed siRNA sequence against the Arylsulfatase B we were able to achieve 41.1% silencing of ARSB mRNA in HPAECs.

The results of the viability assays are presented in Figs. 1 and 2. Both tests showed reduced viability of HPAECs in high DS concentrations. However, the cells incubated without elastin in growth medium showed a biphasic viability response. First the viability of both siARSB transfected and control cells decreased in low concentrations of DS. After reaching the lowest viability at DS 12.5 μL/mL the viability of the cells increased reaching its maximum at 50 μg/mL. Above this concentration the viability of the cells consecutively decreased again. There was also a difference in viability between siARSB and control — at the
25 μg/mL DS concentration the viability of the control group was significantly higher. Other DS concentrations showed also this trend. 50 μg/mL elastin concentration caused a significant reduction viability of HPAECs already at 25 μg/mL with no transient viability gain. The trend of higher HPAECs viability in the control group in comparison to siARSB transfected cells, however statistically non-significant, could also be seen in the experiment with elastin.

The results of the apoptosis measurements as a ratio of corresponding measurements of siARSB transfected cells and their controls are presented in Fig. 3. The cells were analyzed in three subgroups: late apoptotic — labeled with propidium iodide (PI) and FITC Annexin V, early apoptotic — labeled with FITC Annexin V and non-apoptotic — not labeled. The most signal counted in fluorocytometry came from the not labeled cells — non-apoptotic cells (70–75% of whole cell pool —

![Graph](image1.png)

**Fig. 1.** HPAEC viability measured by MTT test without elastin. Influence of various concentrations of dermatan sulfate on the viability of HPAECs transfected with ARSB siRNA without elastin. The corresponding controls are HPAECs transfected with non-targeting siRNA. Data were presented as means and standard errors. Horizontal bars show statistical significant differences with p-values between values at the bar ends.

![Graph](image2.png)

**Fig. 2.** HPAEC viability measured by MTT test at elastin 50 μg/mL. Influence of various concentrations of dermatan sulfate on proliferation and viability of HPAECs transfected with ARBS siRNA in the presence of elastin (50 μg/mL). The corresponding controls are HPAECs transfected with non-targeting siRNA. Data were presented as means and standard errors. Horizontal bars show statistical significant differences with p-values between values at the bar ends.
the differences between all groups were not significant), and to the less extent from the early apoptotic cells labeled with Annexin V solely (about one fourth of the cell pool; the differences between all groups were also not significant). Late apoptotic cells labeled with both Annexin V and IP consisted not more than 2.75% of the counts. There has been no influence found for ARSB silencing on the apoptosis of HPAECs in the majority of elastin/dermatan sulfate constellations. Solely the incubation with elastin of siARSB transfected HPAECs showed the trend to reduce the apoptosis in the absence of dermatan sulfate (the number of late apoptotic cells dropped by 33%); however the consecutive increase of apoptosis (measured by Annexin V and IP) with rising DS concentrations could further be observed — the number of the late apoptotic cells increased by 55% in probes with 150 μg/mL DS in comparison to probes with no DS. Furthermore the observed trend to increase the number of non-apoptotic cells with increasing concentrations of DS without elastin was not significant. The necrotic cells labeled with IP solely counted only for a trace signal in the fluorocytometry (less than 0.5% of the counts) and were omitted in Fig. 3.

The impact of ARSB silencing on the expression of endothelial nitric oxide synthase, natriuretic peptide type C and vascular endothelial growth factor A was presented in Fig. 4. All expression measurements were done in various concentrations of DS with and without the addition of elastin. The experiments revealed that the expression of the endothelial nitric oxide synthase in the case of Arylsulfatase B silencing is significantly reduced (Fig. 4a). Reduction of expression levels of eNOS varied with concentrations of the elastin and dermatan sulfate. In the absence of elastin and dermatan sulfate the level of eNOS expression was reduced by 35%. The addition of DS to the growth medium caused further reduction of the eNOS expression to about 1/3 of the normal level. The eNOS expression in the presence of elastin showed about 50% reduction in growth medium without dermatan sulfate and in the 150 μg/mL DS concentration, and in the moderate concentration of DS (50 μg/mL) the reduction was less — about 40%. The expression patterns of NPPC (Fig. 4b) showed a reduced NPPC expression within DS and in high DS concentrations with transient NPPC expression gain up to 20% in middle DS concentrations both with and without elastin. The expression level of VEGFA (Fig. 4c) in siARSB transfected and control cells revealed an opposite effect on increasing concentrations of dermatan sulfate in the presence and absence of elastin. Whereas VEGFA expression level in siARSB transfected HPAECs decreased in raising concentrations of dermatan sulfate, VEGFA expression in the presence of elastin went up with raising concentrations of dermatan sulfate reaching normal level.

4. Discussion

There is little evidence in the literature on the impact of increased amounts of dermatan sulfate and arylsulfatase B shortage on a single cell function. The majority of descriptions of the MPS VI pathology are limited to presenting the pathological processes in the whole systems of the body or organs [42]. However, explanation of the pathology by the DS interference of the elastin receptor has already been proposed — the excess of DS inhibits the calcium signaling dependent on elastin receptor. Elastin is a glycoprotein of cross-linked 72 kD tropoelastin subunits and is an abundant component of extracellular matrix of the arteries, lung and skin [43]. DS enriched extracellular matrix would falsely indicate that the cell resides in an elastin lacking environment or in environment with low concentration of elastin degradation products — elastin derived peptides (EDP) [33–39], that has been shown to promote endothelial cell migration and tubulogenesis [44].

The cardiovascular system is frequently disease-involved in MPS VI: malfunction of endothelium could lead to some of its presentation — valvular disease, coronary artery disease or pulmonary hypertension [45,46]. The pathogenesis of the pulmonary hypertension in MPS VI patients is unknown. Obstructive and restrictive lung disease in concert with upper airway obstruction leading to obstructive sleep apnea [41] may contribute to the hypoxia induced PH in these patients. Moreover, pathology in the left heart i.e. due to mitral valve malfunction and/or dysfunction of the left ventricle might also cause PH [2,3]. Although these factors may be important for the occurrence of PH in MPS VI, the importance of eventual malfunction of the pulmonary artery endothelial cells cannot be omitted during considering PH etiology. This opinion confirms the results of Kelly et al. who documented a significantly impaired endothelial function of MPS children in a non-invasive test measuring digital reactive hyperemia [47].

Endothelium plays a crucial role in many processes within the vascular bed creating an inner layer directly contacting blood. A potential pathology in endothelial cells would have a negative impact on vessels, heart valves, immune and coagulation processes. Thus, the aim of the study was to investigate how arylsulfatase B deficiency changes the endothelial cells of human pulmonary artery. The real conditions on the molecular level in MPS VI patients are unknown and surely vary between separate subjects. To emulate these conditions and having limited reference data from the literature, we decided to create an endothelial cellular model of the MPS VI disease. We tested siARSB transfected HPAECs in various concentrations of storage material.
DS and exposure to stimulation of the elastin receptor by elastin. According to previously published data [32,36,38] DS should interfere the stimulation of elastin receptor and change the intracellular calcium levels causing multiple functional alteration of cells. Such model has its limitations. The main difference between our model of MPS VI and disease itself consists in the way of reduction of ARSB function. In the case of the MPS VI disease the lesion of the function comes from mutation in ARSB gene causing structural and functional alteration of the enzyme. In the proposed MPS VI model the reduction of the enzyme function comes solely from the reduction of ARSB amount. The kinetics of the gene silencing with siRNA does not allow us to precisely determine the level of enzyme activity in our experiments. However we were able to observe statistically significant changes in most of our experiments. Our MPS VI model consists not only from the silencing of the ARSB gene solely, but we analyzed also the HPAECs’ function in the case of DS and elastin excess. In our opinion the functional changes in MPS VI cells come not only from reduced ARSB function exclusively but also from the excess of DS in the cytoplasm and outside cell environment. Moreover the clinically observed abnormalities in MPS VI patients

**Fig. 4.** Analysis of the impact of siARSB transfection in HPAECs on the expression of (a) — endothelial nitric oxide synthase (eNOS), (b) — natriuretic peptide type C (NPPC), and (c) — vascular endothelial growth factor A (VEGFA) in the presence of elastin and dermatan sulfate. The results show, relative to the control (siRNA non-targeting), change of the gene expression in the case of ARSB silencing with corresponding siRNA. EL/D0/DS 0 — without elastin and dermatan sulfate, EL/D0/DS 50 — without elastin and with 50 μg/mL dermatan sulfate, EL/D0/DS 150 — without elastin and with 150 μg/mL dermatan sulfate, EL/50/DS 0 — with 50 μg/mL elastin and without dermatan sulfate, EL/50/DS 50 — with 50 μg/mL elastin and 50 μg/mL dermatan sulfate, EL/50/DS 150 — with 50 μg/mL elastin and 150 μg/mL dermatan sulfate. Data were presented as means and standard errors. Horizontal bars show statistical significance with p-values between values at the bar ends.
References

[1] E.F. Neufeld, J. Muenzer, The mucopolysaccharidoses, in: C.R. Scriber, A.L. Beaudet, W.S. Sly (Eds.), The Metabolic and Molecular Basis of Inherited Disease, McGraw-Hill, New York, 2001, pp. 3421–3452.

[2] A. Golda, A. Jurecka, V. Opoka-Winiarska, A. Tylik-Szymanska, Mucopolysaccharidoses type VI: a cardiologist’s guide to diagnosis and treatment, Int. J. Cardiol. 167 (1) (2013) 1–10.

[3] A. Golda, A. Jurecka, A. Tylik-Szymanska, Cardiovascular manifestations of mucopolysaccharidoses type VI (Maroteaux–Lamy syndrome), Int. J. Cardiol. 158 (2011) 6–11.

[4] G.N. Leal, A.C. de Paula, C. Leon, C.A. Kim, Echocardiographic study of paediatric patients with mucopolysaccharidoses, Cardiol. Young (2010) 254–261.

[5] I.M. Lang, R. Benza, Pulmonary hypertension: chapters of innovation and tribulation, Eur. Heart J. 33 (2012) 961–968.

[6] M. Humbert, O. Sitbon, G. Simonneau, Treatment of pulmonary arterial hyperten-

sion, Am. J. Respir. Crit. Care Med. 165 (2002) 398–405.

[7] S. Rich, B.H. Brundage, Pulmonary hypertension: a cellular basis for understanding the pathophysiology and treatment, J. Am. Coll. Cardiol. 14 (1989) 545–550.

[8] R.M. Tudor, J.C. Marecki, A. Richter, I. Fijalkowska, S. Flores, Pathology of pulmonary hypertension, Clin. Chest Med. 28 (2007) 23–42 (vi).

[9] R.M. Tudor, B. Groves, D.B. Badescu, N.F. Voelkel, Exuberant endothelial cell growth and elements of inflammation are present in plexiform lesions of pulmonary hyper-

tension, Am. J. Pathol. 144 (1994) 275–285.

[10] S. Hirose, Y. Hosoda, S. Furuya, T. Otsuki, E. Ibeda, Expression of vascular endothelial growth factor and its receptors correlates closely with formation of the plexiform lesion in human pulmonary hypertension, Pathol. Int. 50 (2000) 472–479.

[11] R.M. Tudor, C.D. Cool, M. Yeager, L. Taraseviciene-Stewart, T.B. Ball, N.F. Voelkel, The pathobiology of pulmonary hypertension, Endothelin Clin. Med. 22 (2001) 405–418.

[12] J.A. Vita, Endothelial function, Circulation 124 (2011) e905–912.

[13] B. Rudhraja, R.M. Tudor, F.M. Hanssoua, Endothelial dysfunction in pulmonary hypertension, Circulation 109 (2004) 159–165.

[14] N. Davie, S.J. Hakeen, P.D. Upton, J.M. Polak, M.H. Yacobu, N.W. Morrell, J. Wharton, ET(A) and ET(B) receptors modulate the proliferation of human pulmonary artery smooth muscle cells, Am. J. Respir. Crit. Care Med. 165 (2002) 398–405.

[15] P. Jurazs, D. Courtman, S. Babaie, D.J. Stewart, Role of apoptosis in pulmonary hyper-
tension: from experimental models to clinical trials, Pharmacol. Ther. 126 (2010) 1–16.

[16] S. Sakao, L. Taraseviciene-Stewart, K. Wood, C.D. Cool, N.F. Voelkel, Apoptosis of pul-

monary microvascular endothelial cells stimulates vascular smooth muscle cell growth, Am. J. Physiol. Lung Cell. Mol. Physiol. 291 (2006) 1362–368.

[17] L. Taraseviciene-Stewart, Y. Kasahara, L. Alger, P. Hirth, G. Mc Mahon, J. Wenker, N.F. Voelkel, R.M. Tudor, Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death–dependent pulmonary endothelial cell pro-

liferation and severe pulmonary hypertension, FASEB J. 15 (2001) 427–438.

[18] Q.K. Tran, K. Ohashi, H. Watanabe, Calcium signalling in endothelial cells, Cardiovasc. Res. 48 (2000) 13–22.

[19] R. Busse, A. Mulsch, Calcium-dependent nitric oxide synthesis in endothelial cytosol is mediated by calmodulin, FLETS Bitt. 265 (1990) 133–136.

[20] I. Fleming, J. Baurersachs, B. Fisslthaler, R. Busse, Ca2+–independent activation of the endothelial nitric oxide synthase in response to tyrosine phosphatase inhibitors and fluid shear stress, Circ. Res. 82 (1998) 686–695.

[21] F.A. Andrade, C.B. Restini, M.D. Grando, L.N. Ramalho, L.M. Bendhack, Vascular relax-

ation induced by C-type natriuretic peptide involves the Ca2+/NO-synthase/NO

pathway, PLoS One 9 (2014) e95946.

[22] T. Itoh, N. Nagaya, S. Murakami, T. Fuji, T. Iwase, H. Ishibashi-Ueda, C. Yutani, M. Yamagishi, H. Kimura, K. Kangawa, C-type natriuretic peptide ameliorates monocrotaline-induced pulmonary hypertension in rats, Am. J. Respir. Crit. Care Med. 170 (2004) 1204–1211.

[23] S. Murakami, N. Nagaya, T. Itoh, T. Fuji, T. Iwase, K. Hamada, H. Kimura, K. Kangawa, C-type natriuretic peptide attenuates bleomycin-induced pulmonary fibrosis in mice, Am. J. Physiol. Lung Cell. Mol. Physiol. 287 (2004) L1172–1177.

[24] R.S. Scotland, M. Cohen, P. Foster, M. Lovell, A. Mathur, A. Whatlawa, A.J. Hobbs, C.

type natriuretic peptide inhibits leukocyte recruitment and platelet–leukocyte in-

teractions via suppression of P-selectin expression, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 14452–14457.

[25] K. Doi, T. Ibeda, H. Itoh, K. Ueyama, C. Kodaka, Y. Ogawa, J. Yamashita, T.H. Chun, M. Inoue, K. Masatsugu, N. Sawada, Y. Fukunaga, T. Saito, M. Sone, K. Yamahara, H. Kook, M. Komedaa, M. Ueda, K. Nakao, C-type natriuretic peptide induces redifferentiation of vascular smooth muscle cells with accelerated redifferentiation, Arterioscler., Thromb. Vasc. Biol. 21 (2001) 930–936.

[26] N. Ohno, H. Itoh, T. Ibeda, K. Ueyama, K. Yamahara, K. Doi, J. Yamashita, M. Inoue, K. Masatsugu, N. Sawada, Y. Fukunaga, S. Sakaguchi, M. Sone, T. Yurugi, H. Kook, M. Komedaa, K. Nakao, Accelerated redifferentiation with suppressed thrombogenic property and neointimal hyperplasia of rabbit jugular vein grafts by adenosine–mediated gene transfer of C-type natriuretic peptide, Circulation 105 (2002) 1623–1628.

[27] K. Yamashita, H. Itoh, T.H. Chun, Y. Ogawa, J. Yamashita, N. Sawada, Y. Fukunaga, M. Sone, T. Yurugi-Kohayashi, K. Miyashita, H. Tsujiomi, H. Kook, R. Feil, D.L. Garbers, F. Hofmann, K. Nakao, Significance and therapeutic potential of the natriuretic peptides/CMP/CMP-dependent protein kinase pathway in vascular regeneration, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 3404–3409.

[28] S.S. Arcott, D.W. Lipke, M.N. Gillespie, J.W. Olson, Altersions of growth factor tran-

scripts in rat lungs during development of monocrotaline-induced pulmonary hyper-
tension, Biochem. Pharmacol. 46 (1993) 1086–1091.

Acknowledgments

This research work was supported by a 2012 annual prize and scientific grant of the Polish Cardiac Society.

5 Conclusions

Our results demonstrate that the arylsulfatase B deficient pulmonary artery endothelial cell is alternated and differently reacts to factors stim-

ulating elastin receptor. The results of this research can contribute to the evaluation of further studies on endothelial dysfunction aiming to pro-

pose new treatment options and to reduce elevated cardiovascular mor-

tality in MPS VI patients.
[29] C. Partovian, A. Ladoux, S. Eddahibi, E. Teiger, B. Raffestin, C. Frelin, S. Adnot, Cardiac
and lung VEGF mRNA expression in chronically hypoxic and monocrotaline-treated
rats, Chest 114 (1998) 455–465.
[30] A.I. Campbell, Y. Zhao, R. Sandhu, D.J. Stewart, Cell-based gene transfer of vascular
endothelial growth factor attenuates monocrotaline-induced pulmonary hyperten-
sion, Circulation 104 (2001) 2242–2248.
[31] R.L. Juliano, S. Haskill, Signal transduction from the extracellular matrix, J. Cell Biol.
120 (1993) 577–585.
[32] C.Q. Lin, M.J. Bissell, Multi-faceted regulation of cell differentiation by extracellular
matrix, FASEB J. 7 (1993) 737–743.
[33] R.P. Mechem, A. Hinek, R. Entwistle, D.S. Wrenn, G.L. Griffin, R.M. Senior, Elastin
binds to a multifunctional 67-kilodalton peripheral membrane protein, Biochemis-
try (Mosc) 28 (1989) 3716–3722.
[34] A. Hinek, D.S. Wrenn, R.P. Mechem, S.H. Barondes, The elastin receptor: a
galactoside-binding protein, Science 239 (1988) 1539–1541.
[35] A. Hinek, Biological roles of the non-integrin elastin/laminin receptor, Biol. Chem.
377 (1996) 471–480.
[36] J. Yannariello-Brown, U. Wewer, L. Liotta, J.A. Madri, Distribution of a 69-kD laminin-
binding protein in aortic and microvascular endothelial cells: modulation during cell
attachment, spreading, and migration, J. Cell Biol. 106 (1988) 1773–1786.
[37] A. Hinek, M. Rabinovitch, F. Keeley, Y. Okamura-Oho, J. Callahan, The 67-kD elastin/
laminin-binding protein is related to an enzymatically inactive, alternatively spliced
form of beta-galactosidase, J. Clin. Invest. 91 (1993) 1198–1205.
[38] A. Hinek, J. Boyle, M. Rabinovitch, Vascular smooth muscle cell detachment from
elastin and migration through elastic laminae is promoted by chondroitin sulfate-
induced “shedding” of the 67-kDa cell surface elastin binding protein, Exp. Cell
Res. 203 (1992) 344–353.

[39] M.P. Jacob, T. Fulop Jr., G. Foris, L. Robert, Effect of elastin peptides on ion fluxes in
mononuclear cells, fibroblasts, and smooth muscle cells, Proc. Natl. Acad. Sci. U. S. A.
84 (1987) 995–999.
[40] M.R. Latex, J.J. Rossi, D.L. Ouellet, RNAi and small interfering RNAs in human disease
therapeutic applications, Trends Biotechnol. 28 (2010) 570–579.
[41] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative
CT method, Nat. Protoc. 3 (2006) 1101–1108.
[42] V. Valayannopoulos, H. Nicoly, P. Harmatz, S. Turbeville, Mucopolysaccharidosis VI,
Orphanet J. Rare Dis. 5 (2010) 5.
[43] M.R. Hayden, J.R. Sowers, S.C. Tyagi, The central role of vascular extracellular matrix
and basement membrane remodeling in metabolic syndrome and type 2 diabetes: the
matrix preloaded, Cardiovasc. Diabetol. 4 (2005) 9.
[44] A. Robinet, A. Fahern, H. Cauchard, E. Huet, L. Vincent, S. Lorimier, F. Antonicelli, C.
Soria, M. Crepin, W. Hornebeck, C. Bellon, Elastin-derived peptides enhance angio-
genesis by promoting endothelial cell migration and tubulogenesis through upreg-
ulation of MT1-MMP, J. Cell Sci. 118 (2005) 343–356.
[45] A. Jurecka, E. Zakharova, L. Cimbalistiene, N. Gusina, A. Kulpanovich, A. Golda, V. Opoka-
Winarska, E. Piotrowska, E. Voskoboeva, A. Tylik-Szymanska, Mucopolysaccharidosis
type VI: a predominantly cardiac phenotype associated with a homozygosity for
p.R152W mutation in the ARSB gene, Am. J. Med. Genet. A 161 (2013) 1291–1299.
[46] A. Jurecka, A. Golda, V. Opoka-Winiarska, E. Piotrowska, A. Tylik-Szymanska,
Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) with a predominant-
ly cardiac phenotype, Mol. Genet. Metab. 104 (2011) 695–699.
[47] A.S. Kelly, A.M. Metzig, J. Steinberger, E.A. Braunlin, Endothelial function in children
and adolescents with mucopolysaccharidosis, J. Inherit. Metab. Dis. 36 (2013)
221–225.