SPANDIDOS PUBLICATIONS

Expression of TGF-ß1 and its receptor genes (TßR I, TßR II, and TßR III-betaglycan) in peripheral blood leucocytes in patients with idiopathic pulmonary arterial hypertension and Eisenmenger's syndrome

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Abstract. Idiopathic pulmonary arterial hypertension (IPAH) is characterized by smooth muscle cell, endothelial cell, and fibroblast hypertrophy and an increase in extracellular matrix volume in pulmonary precapillary arterioles. These features lead to a gradual increase of pulmonary vascular resistance, right-heart failure, and premature death. Bone morphogenetic protein receptor type 2 (BMPR-2) gene mutations have been identified to cause IPAH. BMPR-2 receptor mutation results in BMP signalling pathway termination and leads to disturbed growth and differentiation of pulmonary circulation cells. Transforming growth factor (TGF)-ß1 inhibits the migration and proliferation of endothelial and smooth muscle cells, and stimulates their differentiation, thus it has antiinflammatory and immunosuppressive properties, inhibiting vascular remodeling and is responsible for extracellular matrix production. The aim of this study was to analyse the profile of TGF-ß1 and the expression of its receptor (TßR I, TßR II and TßR III-betaglycan) genes in IPAH and in secondary forms of pulmonary arterial hypertension [Eisenmenger's syndrome (ES) patients]. Twenty-one patients with IPAH (2 men), 12 ES patients, and 10 healthy controls were enrolled in the study. QRT-PCR analysis of the transcriptive activity of TGF-ß1 and its receptor genes was performed with each patient. There were differences in receptor gene expression among the patient groups. The highest expression was observed in Eisenmenger syndrome patients (approximately 5-to 8-fold increase). There was a negative correlation between the gene expression of TGF-ß1 and that of its receptors, and a positive correlation between TßR II and TßR III in healthy controls. In IPAH patients a positive correlation between TGF-ß1 and TßR I was found. There was a difference in expression of TGF-ß1/receptor gene ratios and expression of receptor gene ratios between the examined groups. The differences in expression between IPAH and ES patients might suggest the role of these cytokines in IPAH pathogenesis. A disturbed proportion of expression of TGF-ß1 and receptor genes in IPAH patients might be one of the pathogenetic factors of the disease.

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Introduction

Idiopathic pulmonary arterial hypertension (IPAH) is characterized by smooth muscle cell, endothelial cell, and fibroblast hypertrophy and an increase in extracellular matrix volume in pulmonary precapillary arterioles. Moreover, plexogenic lesion formation and *in situ* thrombosis can occur (1-3). These features lead to a gradual increase of pulmonary vascular resistance, right-heart failure, and premature death. The mean survival time from the onset of symptoms in untreated patients does not exceed 5 years, and in general it ranges from 2.5 to 4 years (4-6).

Several studies have shown that IPAH is in some cases genetically determined. It is inherited in an autosomal dominant manner with 10-20% penetration. Familial pulmonary arterial hypertension occurrs in approximately 6% of cases (7-9).

The bone morphogenetic protein receptor type 2 (BMPR 2) gene [BMPR 2 mutations, coding for a receptor member of the transforming growth factor (TGF-ß) superfamily] (Fig. 1), has been identified to cause IPAH (10-16). Its main influence on blood vessels is to inhibit vascular smooth muscle cell proliferation and induce their apoptosis, and to protect endothelial cells and their respective progenitor cells. TGF-ß1 is expressed in endothelial cells, smooth muscle cells, leucocytes and epithelial cells. It inhibits the migration and proliferation of these cells and stimulates their differentiation,

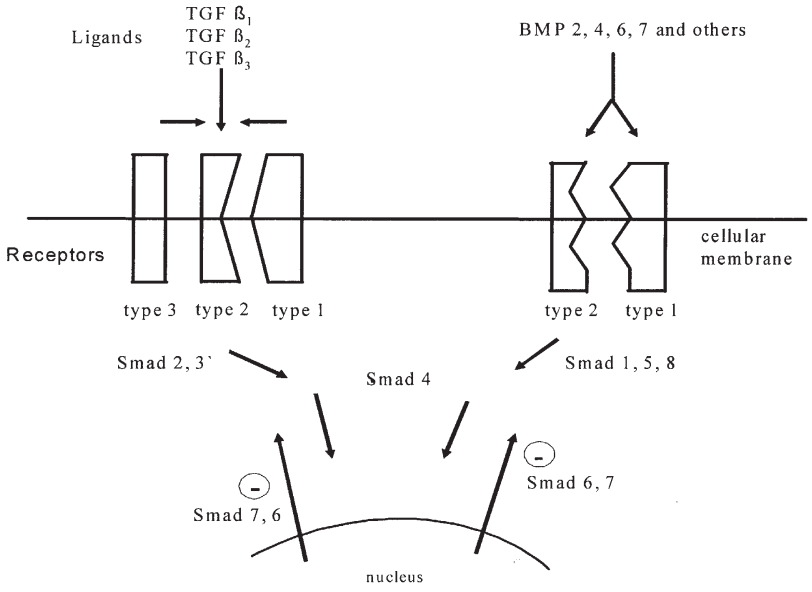

Figure 1. Pattern of action of the TGF-ß superfamily.

thus it has antiinflammatory and immunosuppressive properties, inhibits vascular remodeling and is responsible for extracellular matrix production (17-19).

There are two types of BMP receptors: type 1 (two subtypes BMPR1a and BMPR1b) and type 2 (BMPR 2). There are two receptors for TGF-ß, type I and type II (TßR I and TßR II), and two auxilliary type III receptors, endoglin and betaglycan. An activated type II receptor is responsible for type I receptor phosphorylation with modulatory influence of betaglycan and endoglin. Both receptors, betaglycan and endoglin, are present in microcirculation and endothelium, wherein they act in an opposite manner; betaglycan enhances TGF-ß function and endoglycan inhibits it (20-24). Incorrect BMP and TGF-ß signalling pathway activation seems to have a significant role in pulmonary hypertension pathogenesis. BMPR 2 receptor mutation results in BMP signalling pathway termination and leads to disturbed growth and differentiation of pulmonary circulation cells (25). It is shown that other components of the TGF-ß superfamily are also malfunctioning. Vascular smooth muscle cells harvested from diseased pulmonary vessels exhibit incorrect activation to either BMP or TGF-ß1 stimulation. They start proliferating after TGF-ß1 and BMP stimulation, resulting in hypertrophy and vascular cell remodeling. In parallel, the proapoptotic activity of these cytokines is abolished (26-28). The data indicate a significant role of the TGF-ß superfamily and its receptors in pulmonary arterial hypertension pathogenesis. It is not known whether the activation of some of the TGF-ß superfamily compound is primary in origin, secondary to altered pulmonary haemodynamics, caused by other factors, or whether the modified cell phenotype is responsible for pathologic activation of the cell.

The aim of the study was to assess the expression of TGF-ß1 and its TßR I, TßR II, and TßR III-betaglycan receptor genes, associated with angiogenesis, in IPAH patients. The comparison with Eisenmenger's syndrome (ES) and healthy control patients may be helpful to assess the impact of these pathways in IPAH pathogenesis.

Materials and methods

Thirty-five patients with pulmonary arterial hypertension were enrolled into the study; 22 with IPAH (19 women) aged 12-69 years, with a mean age of 38 years, and 13 patients with Eisenmenger's syndrome (7 women), aged 20-59 years, with a mean age of 35.5 years. The control group comprised 10 healthy volunteers (5 women), with a mean age of 34 years.

The clinical characteristics of IPAH patients is shown in Table I. A diagnosis of idiopathic pulmonary arterial hypertension was established on the basis of generally accepted criteria, including cardiac catheterization (29). Enrolled patients were not treated with 'modern' pharmacological agents; PGI-2 analogues, endothelin receptor blockers or PDE-5 inhibitors. The clinical characteristics of patients with Eisenmenger's syndrome is shown in Table II. At the time of blood sampling, ES patients were not administered any drugs.

Blood for genetic studies from IPAH patients was drawn during hospital stay in which haemodynamic examination was performed. Blood from ES patients was drawn during out-patient visits.

Molecular studies were performed in the Department of Molecular Biology and Medical Genetics, Silesian Medical University, Sosnowiec. The study protocol was accepted by the local Ethics Committee.

Extraction of total RNA. Total RNA was isolated from the whole blood sample (A&A Biotechnology). The quality of the RNA was assessed by gel electrophoresis and quantitative analysis of RNA extracts was performed spectrophotometrically using GeneQuant II (Pharmacia Biotech).

mRNA quantification by real-time reverse transcription polymerase chain reaction. The quantitative analysis was carried out with the use of an ABI PRISM 7000 sequence detector (Applied Biosystems). The quantity of PCR amplicons was

'linical and haemodynamic profile of patients with IPAH.

Patient	Sex	Age at onset (years)	Age at examination (years)	mPAP (mm Hg)	PCWP (mm Hg)	CI (l x min^{-2} x m^{-2})	PVR (Wood's units)
1	M	64	69	60.6	10.0	2.00	25.30
2	F	26.5	26.8	41.7	0.0	1.41	29.30
3	F	41.1	43	50.7	5.0	3.02	15.10
4	F	20	22	531	9.0	3.49	12.60
5	F	48	54	59.2	13.0	1.42	32.20
6	F	43	43	53.7	12.0	2.38	17.50
7	F	45	57	56.7	9.0	2.44	19.50
8	F	58	60	54.3	3.0	1.62	31.70
9	F	9	12	51.6	10.0	1.82	22.40
10	F	25	59	32.7	12.0	2.82	7.32
11	F	37	43	31.7	4.0	5.35	5.16
12	M	10	36	95.7	13.0	1.96	42.00
13	F	3	6	96.7	13.0	1.20	69.70
14	F	17	24	62.3	7.0	2.69	20.50
15	F	31	51	48.3	1.0	2.02	23.20
16	F	31	41	59.3	12.0	2.78	17.00
17	F	39	45	65.7	6.7	1.96	30.00
18	F	27	52	34.3	3.0	1.89	16.50
19	F	17	21	93.3	10.0	3.37	24.70
20	F	43	48	19.3	0.0	5.28	3.66
21	F	29	35	38.4	5.0	3.29	10.00
22	M	23	23	96.7	10.0	1.53	56.30

mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CI, cardiac index; PVR, pulmonary vascular resistance.

determined after each round of amplification, using the fluorescent dye SYBR-Green I (Qiagen), which binds double-stranded DNA. The quantity of amplified cDNA fragments was determined from the Ct value (threshold value of fluorescence) with a reference to the standard curve generated by amplification of five known concentrations of the ß-actin gene ($1x10^3$-$2x10^4$ copies; TaqMan template reagent, ß-actin control reagent kit; Applied Biosystems, USA).

TGF-ß1 and receptors TßR I, TßR II, and TßR III. The nucleotide sequences of the PCR primers used to assay gene expression were designed using the computer program Primer Express™ Version 1.0 ABI PRISM: TGF-ß1, 5' TGAACCG GCCTTTCCTGCTTCTCATG 3' (forward primer) and 5' GC GGAAGTCAATGTACAGCTGCCGC 3' (reverse primer); TßR I, 5' ACTGGCAGCTGTCATTGCTGGACCAG 3' (forward primer) and 5' CCTGAGCCAGAACCTGACGTTG TCATATCA 3' (reverse primer); TßR II, 5' GGCTCAACC ACCAGGGCATCCAGAT 3' (forward primer) and 5' CTC CCCGAGAGCCTGTCCAGATGCT 3' (reverse primer); and TßR III, 5' ACCGTGATGGGCATTGCGTTTGCA 3' (forward primer) and 5' GTGCTCTGCGTGCTGCCGATGCTGT 3' (reverse primer). The RT-PCR reaction mixture of a total volume of 50 μl contained 25 μl 2X QuantiTec SYBR-Green

Table II. Profile of patients with Eisenmenger's syndrome.

Patient	Sex	Age at examination (years)	Diagnosis
1	F	59	ASD t II
2	F	21	TGA, common ventricle
3	F	20	VSD
4	M	41	ASD t II, TAPVD
5	F	23	VSD, aorto-pulmonary window
6	M	45	VSD
7	M	31	VSD
8	F	39	VSD, PDA
9	F	27	VSD, PDA
10	M	47	VSD
11	M	32	VSD
12	F	52	VSD
13	M	28	VSD

ASD t II, atrial septal defect t II; TGA, transposition of great arteries; VSD, ventricular septal defect; PDA, patent ductus arteriosus; TAPVD, total anomalous pulmonary drainage.

Table III. Expression of the TGF-ß1 gene and genes of its receptors, type I, II, and III-betaglycan in examined groups.

	TGF-ß1	TßR I	TßR II	TßR III
	(copies of mRNA per 1 ug RNA x 10^3)			
Control (n=10)				
Mean	19.021	12.96	19.01	12.86
Median	16.21	12.61	17.04	11.61
IQR	9.98	7.34	13.52	5.06
IPAH (n=22)				
Mean	22.03	23.67	16.01	19.62
Median	16.78	14.80	13.93	17.70
IQR	19.49	12.08	11.36	22.65
ES (n=13)				
Mean	13.41[a]	63.88[b]	105.88[b]	106.10[b]
Median	11.58	53.02	101.73	104.64
IQR	6.13	50.33	12.73	33.50

Mann-Whitney U test: [a]p<0.05 (ES: control), [b]p<0.001 (ES: control, IPAH).

Table IV. Correlations between the gene of TGF-ß1 and its receptor genes.

	Control		IPAH		ES	
	R	p	R	p	R	p
TGF-ß1-TßR I	-0.358	NS	0.431[a]	0.044[a]	0.33	NS
TGF-ß1-TßR II	-0.685[a]	0.029[a]	0.17	NS	-0.06	NS
TGF-ß1-TßR III	-0.685[a]	0.029[a]	0.377	NS	0.11	NS
TßR I-TßR II	0.321	NS	-0.013	NS	-0.165	NS
TßR I-TßR III	0.454	NS	0.156	NS	0.088	NS
TßR II-TßR III	0.867[a]	0.001[a]	-0.072	NS	-0.297	NS

Spearman's rank (R) test: [a]p<0.05.

RT-PCR master mix composed of HotStarTaq DNA polymerase, QuantiTec SYBR-Green RT-PCR buffer containing Tris-HCl, KCl, $(NH_4)_2SO_4$, 5 mM $MgCl_2$, pH 8.7, dNTP mix, fluorescent dye SYBR-Green I, and passive reference dye ROX mixed with 0.5 μl QuantiTect RT mix (Omniscript reverse transcriptase, Sensiscript reverse transcriptase) (QuantiTect SYBR-Green RT-PCR kit; Qiagen), forward and reverse primers (each at a final concentration of 0.5 μM), mRNA template, and RNase-free water to a final volume of 50 μl.

Standards of ß-actin. ß-actin DNA was amplified using two oligonucleotide primers: 5' TCACCCACATGTGCCCATCT ACGA 3' (forward primer) and 5' CAGCGGAACCGCTCAT TGCCAATGG 3' (reverse primer). The PCR reaction mixture of a total volume of 50 μl contained 25 μl of 2X QuantiTect SYBR-Green PCR master mix [HotStarTaq DNA polymerase, QuantiTect SYBR-Green buffer containing Tris-HCl, KCl, $(NH_4)_2SO_4$, 5 mM $MgCl_2$, pH 8,7, dNTP mix, fluorescent dye SYBR-Green I, passive reference dye ROX] (QuantiTect SYBR-Green PCR kit; Qiagen), forward and reverse primers (each at a final concentration of 0.3 μM), DNA template and RNase-free water to a final volume of 50 μl. Reverse transcription was carried out at 50˚C for 30 min. After activation of the HotStarTaq DNA polymerase and deactivation of reverse transcriptases at 95˚C for 15 min, subsequent PCR amplification consisted of denaturation at 94˚C for 15 sec and annealing at 60˚C for 30 sec (45 cycles). Extension was carried out at 72˚C for 10 min.

Sequence specificity of amplimers. Melting temperatures, assessed by a SYBR-Green I dissociation assay (Dissociation Curve Software; Applera Biosystems), were compared with theoretical melting temperatures assessed by computer software (http://www.promega.com/biomatch/calc). The PCR products and molecular weight marker pBR322/Bsu RI (Fermentas) were separated on 8% polyacrylamide gel and visualised using silver staining (LKB-Pharmacia). The length of the amplified fragments was assessed by analysis with Biotec-Fisher BAS-SYS 1D software.

Statistical analysis. Data presented are the arithmetic mean with standard deviation (SD) or median with interquartile range (IQR). Statitistical analysis used Mann-Whitney U test for comparison of data without normal distribution. Correlation was assessed with Spearman's rank test. p<0.05 was considered significant.

Results

Mean values of the TGF-ß1 gene and its receptors' expression are shown in Table III. Expression of TGF-ß1 in the IPAH group was comparable with that of the control group. TGF-ß1 expression in the ES group was significantly lower in comparison to that of the healthy controls.

Mean value of TßR I gene expression in the IPAH group was insignificantly higher in comparison to that of the control group. TßR I gene expression in ES patients was almost three times greater than that in the IPAH group and almost five times greater than that in the control group (p<0.001).

Mean value of TßR II gene expression in the IPAH group was comparable with that in the control group. Expression of the TßR II gene in ES patients was five times higher than that in the control and IPAH groups (p<0.001).

Mean value of TßR III gene expression in the IPAH group was insignificantly higher in comparison to that in the control group. TßR III gene expression in ES patients was eight-fold higher in comparison to that in the control group and five-fold higher in comparison to that in the IPAH group (p<0,001).

The results of correlation analysis of studied parameters are shown in Table IV. TGF-ß1 gene expression in the control group negatively correlated with the expression of its receptor genes (correlations with TßR II and TßR III were statistically significant). Expression of the receptor genes positively correlated (correlation between TßR II and TßR III was significant). TGF-ß1 gene expression positively correlated with TßR I and TßR II gene expression (borderline significance of

Ratios of receptor gene expression in examined

	TßR I/TßR II	TßR III/TßR II	TßR III/TßR I
Control (n=10)			
Mean	0.74	0.72	1.17
Median	0.63	0.74	1.20
IQR	0.51	0.23	0.74
IPAH (n=22)			
Mean	2.63[a,b]	1.76[a]	1.41[b]
Median	1.13	1.28	0.86
IQR	1.52	1.93	1.03
ES (n=13)			
Mean	0.64	1.06[c]	2.75[c]
Median	0.51	1.00	1.60
IQR	0.57	0.65	0.69

Mann-Whitney U test: IPAH/control, [a]0.07>p>0.05; IPAH/ES, [b]p<0.05; ES/control: [c]p<0.05.

Table VI. Ratios of expression of TGF-ß1 and its receptors in examined groups.

	TGF-ß1/ TßR I	TGF-ß1/ TßR II	TGF-ß1/ TßR III
Control (n=10)			
Mean	2.14	1.31	1.70
Median	1.34	1.03	1.37
IQR	0.77	0.82	1.20
IPAH (n=22)			
Mean	1.28[c]	1.75[c]	1.43[c]
Median	1.16	1.42	1.01
IQR	0.99	1.39	1.29
ES (n=13)			
Mean	0.30[f]	0.13[f]	0.13[f]
Median	0.19	0.10	0.11
IQR	0.16	0.06	0.06

Mann-Whitney U test: IPAH/control, NS; IPAH/ES, [a]p<0.05, [b]p<0.01, [c]p<0.001; ES/control, [d]p<0.05, [e]p<0.01, [f]p<0.001.

correlation with TßR III) in IPAH patients. It is apparent in IPAH patients that the correlation between TGF-ß1 and its receptors was positive (in contrast to such correlation in the control group). Moreover there is not any association among receptor gene expression neither in IPAH nor in ES patients.

For further analyses we transformed the results of gene expression by dividing TGF-ß1 expression by expression of receptors and the latter by each other. Results are shown in Tables V and VI. There was relative overexpression of the TßR III gene compared to TßR I and TßR II in IPAH patients in comparison to control patients. TßR II was relatively lower in comparison to TßR I gene expression.

The proportions of gene expression of TßR I/TßR II and TßR III/TßR II had borderline significance in comparison to the control group. There was relative overexpression of TßR III compared to TßR I in the ES group, when compared to the control group. The proportions of gene expression of TßR III/ TßR I and TßR III/TßR II were significantly different between the ES and the control group. There were also significant differences between the ES and IPAH group, when proportions of TßR I/TßR II and TßR III/TßR I were analysed.

The proportions of TGF-ß1 gene expression and receptor gene expression were comparable in the control and IPAH groups. They were much higher when compared to the ES group.

Discussion

IPAH is a disease of unclear ethiology. The discovery of an association between BMPR 2 gene mutation and the occurrence of pulmonary arterial hypertension has led to further investigation of expression of receptors for this gene, and other genes of the BMP pathway and TGF-ß superfamily (24-28). Data on the impact of TGF-ß1 and its receptors in development and differentiation of pulmonary arteries and lung tissue is abundant, in contrast to that referring to their role in pulmonary arterial hypertension pathogenesis.

Some studies on expression of genes associated with pulmonary artery hypertension were carried out on lung tissue. Geraci *et al* (30) have found increased expression of genes encoding ion channels and oncogenes, decreased expression of genes encoding kinases and phosphatases, and genes responsible for apoptosis. Bull *et al* (31) were the first to analyse gene expression in peripheral blood leucocytes from patients with different types of pulmonary artery hypertension. There were differences in expression of genes responsible for pulmonary artery hypertension between patients with idiopathic and secondary forms of pulmonary hypertension. The authors did not analyse the genes from the TGF-ß superfamily. They have proved that material for genetic analysis from luecocytes is suitable for the assessment of genes implicated in pulmonary hypertension pathogenesis.

BMPR 2 gene expression in lung tissue of IPAH patients is decreased, independently of possible mutations (24,26,27). Several components of the TGF-ß1 superfamily were studied. There is no data in the literature on TGF-ß1 and its receptor genes' expression in IPAH patients.

Pathogenesis of IPAH and ES is different. It is accepted, that BMPR 2 gene mutation, resulting in down-regulation of the inhibitory influence of BMP on resistance arteriole smooth muscle cells, is one of the causes of IPAH. Disturbed apoptosis and secondary selection of cells, characterized by uncontrolled growth, leads eventually to pulmonary hypertension (32,33).

In pulmonary arterial hypertension associated with congenital heart disease the principal cause leading to pulmonary vascular remodeling is chronically increased blood flow.

The shear stress causes injury to the vascular endothelium and stimulates vascular cells to produce an array of pro- and anti-angiogenic substances. This results in hypertrophy and remodeling of arteries. The TGF-ß superfamily has a strong impact on these processes. When pulmonary pressure and resistance approach systemic levels, the direction of the flow becomes bidirectional or right-to-left (Eisenmenger's syndrome).

Mata-Greenwood *et al* (34) analyzed the expression of TGF-ß genes in consecutive stages of hyperkinetic pulmonary hypertension. There was a low expression level of TGF-ß1 in initial and final phases of pulmonary artery remodeling, but in proportion to increased blood flow, TGF-ß1 and type I receptor (ALK 1) gene expression increased. Expression of type II receptor was not changed during the observation and was comparable to that of the control group. Expression of type I receptor (TßR I) was decreased in the phase of most intensive arterial remodeling, in reverse to TGF-ß1, ALK 1, and the control group. Later on, in the course of the disease its expression was similar to that of the control group. Gao *et al* (35) studying patients with left-to-right shunt, but without Eisenmenger's syndrome, found a correlation between lesions in pulmonary arterioles, increased TGF-ß1 expression in this location, and the occurrence of pulmonary hypertension. There were not arteriolar lesions or increased expression of TGF-ß1 in patients without pulmonary hypertension. This confirms secondary changes in TGF-ß1/receptor expression due to chronically increased blood flow. Other authors report that TGF-ß1 expression is elevated in the early phases of secondary pulmonary arterial hypertension, and decreases in later stages (36,37).

The influence of TGF-ß1 on development of pulmonary vessels is complex. It depends on the stage of development, type of tissue, cellular origin, and regulatory factors influencing the development process (34,38). In normal conditions, TGF-ß1 has inhibitory properties on proliferation and migration of smooth muscle and endothelial cells. Morrell *et al* (26) reported a paradoxical influence of TGF-ß1 and BMP 2, 4, and 7 on smooth muscle cells in pulmonary vessels in IPAH patients. There is not only resistance of smooth muscle cells to the antiproliferative action of BMP and TGF-ß1, but also stimulation to proliferate in IPAH patients, in contrast to healthy controls and patients with secondary pulmonary hypertension. This might be the result of BMPR 2 gene mutations, however it explains only resistance to BMP. TGF-ß1 is not a ligand for BMP receptors, thus the consecutive sequele is more extensive. Cellular proliferation resulting from TGF-ß1, BMP 2, 4, and 7 stimulation, may result from the excess of inhibitory proteins Smad 6 and 7. The damage of BMP pathway receptors (BMPR 2 gene mutation) leads to cellular accumulation of Smad 6 and, to a lesser extent, Smad 7. Smad 6 has greater affinity to BMP pathway, however when present in excess, it inhibits TGF-ß pathway, similarly to Smad 7 (39-43). Decreased activation of Smad 1 proteins is observed in BMPR 2 gene mutation carriers (44). In parallel to inhibition of BMP and TGF-ß pathways by Smad proteins, BMP stimulates alternative pathways via MAPK p38 kinases, and thus promotes proliferation (45,46).

The diverse effect of TGF-ß1 on TßR I and ALK 1 receptors is important in angiogenesis. TGF-ß1 has greater affinity to TßR I in comparison to ALK 1. Thus in low concentrations it stimulates primarily the TßR I receptor, inhibiting angiogenesis, whereas in high concentrations, it stimulates ALK 1, enhancing angiogenesis (19,34,47-49). Takeda *et al* (24) reported different effects of TGF-ß1 on vascular smooth muscle cells extracted from IPAH patients. TGF-ß1, in low concentrations, stimulated the growth of pulmonary arteries, but in higher concentrations the growth was inhibited.

The degree of disease progression is also an important factor. The patients examined in our study were in the late advanced symptomatic stage of the disease. Botney *et al* (18) reported the highest activity of the TGF-ß3 isoform, lower activity of TGF-ß2, and absent activity of TGF-ß1 in pulmonary arteries extracted from IPAH patients. The transcriptional activity of TGF-ß1 reported in our study of IPAH patients is comparable to that reported in the control group. The aforementioned differences in TGF-ß1 activity were reported also for ES patients (34).

The role of TßR II in pathogenesis of IPAH is not clear. Under physiologic conditions it is necessary to activate the type I receptor. A prominent role of TßR II in the antiproliferative function of TGF-ß1 was documented. Sankar *et al* (19) reported that decreased expression of TßR II in normal endothelial cell culture inhibits binding of TGF-ß1 to TßR I and leads to promotion of proliferation. A relative increase in TßR I expression, leading to extracellular matrix proliferation, was reported in parallel. A significant decrease in betaglycan expression, correlating with decreased expression of TßR II, was reported also. The positive correlation between betaglycan and TßR II in our control group is in accordance with the aforementioned observations (Table IV). Geraci *et al* (30) reported a decrease in betaglycan and TßR II expression in patients with IPAH. The decrease of expression of type II receptor decreases also antiproliferative properties of TGF-ß1 in neoplasms. It was shown moreover that re-expression of TßR II slows neoplastic cell growth due to enhanced susceptibility to TGF-ß1 (50-54). Yeager *et al* (55) reported somatic mutations in TßR II and BAX genes (the latter are responsible for apoptosis) in cells from plexiform lesions in IPAH patients. It is noteworthy that endothelial cell proliferation in IPAH is monoclonal, in contrast to secondary forms of pulmonary hypertension. It is probable that somatic mutations are the result of the disease rather than its cause. The occurrence of such mutations and monoclonal proliferation of endothelial cells make the pathology of IPAH similar to that of neoplasms.

Atkinson *et al* (27) studied the expression of BMP and TGF-ß1 receptors and their location in lung tissue of patients with IPAH, ES, and pulmonary hypertension due to pulmonary embolism and secondary to connective tissue disease. The authors reported that expression of type II receptor for TGF-ß was similar in studied groups and control subjects. Decreased expression of the BMPR 2 gene in the same areas was reported also. These data are consistent with our results in the IPAH group, although they are contrary to the results observed in our ES group.

Richter *et al* (56) studied the expression of TGF-ß receptors (TßR I and TßR II) and Smad (1,5,8,4,2,3) proteins in pulmonary artery endothelium from IPAH patients. They

high expression of receptor genes and Smad proteins and IPAH patients' endothelial cells. The decrease in their amount may lead to excessive growth and disturbed differentation of endothelial cells in plexiform lesions.

The role of auxilliary receptors (including betaglycan) in IPAH patients has not been established. They can influence the function of the other receptors. The presence of betaglycan in microcirculation cells was reported by Morello *et al* (57) and Wong *et al* (22). The studies of cellular cultures revealed that betaglycan is located in the endothelium, the place where the highest accumulation of capillaries is present and where angiogenesis occurs. Betaglycan is absent in the endothelium of macrocirculation. Betaglycan plays a role in cellular differentiation. Indifferentiated cells in the fetal period have high expression of betaglycan. During the differentiation, betaglycan expression decreases in *in vitro* experiments (58). Letamendia *et al* (21) in the experiment on animal myoblasts found that an increase of betaglycan expression enhances the inhibitory effect of TGF-ß1 on cellular proliferation. Similar results were obtained by other authors (19,22).

Philip *et al* (59) reported that betaglycan in susceptible cells enhances the activity of TGF-ß, however in other cells it has inhibitory properties. The mechanism of its action was explained by Eickelberg *et al* (60); its re-expression in cells in which it was absent is associated with an altered function of this receptor and TGF-ß1 affinity is decreased. This is caused by the altered structure of glycosaminoglycan, a domain responsible for its function. Sankar *et al* (61) in an aortic cell culture (naturally unable to produce the betaglycan) reported that, after addition of betaglycan, production of type I and II was decreased, thus TGF-ß1 affinity was decreased and furthermore its antiproliferative activity was attenuated. Thus we might assume that relative overexpression of TßR III (betaglycan) compared to TßR II in IPAH patients may lead to attenuation of the antiproliferative activity of TGF-ß1. Moreover the disturbed proportion in the expression of each of the TGF-ß1 receptors may induce and cause progression of IPAH.

BMP receptors form homo- and hetero-complexes, and TGF-ß receptors probably form similar complexes (62,63). Thus the net effect of TGF-ß1's function not only determines the type of receptor, but also the number of associated receptor units. Gilboa *et al* (64) suggest that the greatest role of dimers of TGF-ß1 receptors, especially type I and II receptors, is in signal transferring. A change in the number of associated receptor units results in different cellular answers to TGF-ß1 and may explain its pleiotropic properties.

According to our results and published studies, it seems probable that the dominant impact of the TGF-ß1/receptor pathway in induction and progression of IPAH depends on the proportion of expression of each particular gene and not on their net amount. However this suggestion warrants further studies.

In conclusion, there are changes in expression of TGF-ß1/receptor pathway genes in IPAH patients. The differences in expression between IPAH and Eisenmenger syndrome patients might suggest the role of these cytokines in IPAH pathogenesis. A disturbed proportion of expression of TGF-ß1 and receptor genes in IPAH patients might be one of the pathogenetic factors of the disease.

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