The Transforming Growth Factor-Beta Signaling Pathway Involvement in Cardiovascular Lesions in Mucopolysaccharidosis-I

S. Yano • C. Li • Z. Pavlova

Received: 27 February 2012 / Revised: 27 February 2012 / Accepted: 6 March 2012 / Published online: 18 April 2012
© SSIEM and Springer-Verlag Berlin Heidelberg 2012

Abstract Mucopolysaccharidoses (MPS) are a group of genetic disorders due to deficiency of lysosomal enzymes resulting in impaired glycosaminoglycan metabolism. All types of MPS can present with cardiovascular manifestation, although MPS-I, II, and VI seem to have more severe involvement than the other types. Enzyme replacement therapy (ERT) is available for MPS-I, II, and VI. Cardiovascular changes including hypertrophic cardiomyopathy, thickened valvular lesions, and coronary artery lesions often poorly respond to ERT and are well known as leading causes of death in patients with MPS-I. The mechanisms to cause these changes in MPS-I have not been well characterized. Immunohistopathological studies were conducted on the cardiac specimens from a patient with MPS-I who died due to sudden cardiac failure. Phosphorylated Smad2 staining showed hyperactive transforming growth factor-beta (TGF- β) signals in the intimal layer with myointimal proliferation causing stenosis in the coronary arteries as well as in the thickened endocardium

and in the myocardial cells. TGF- β is involved in the pathogenesis of cardiovascular diseases including hypertrophic cardiomyopathy and vascular atherosclerosis. The primary mechanisms to cause hyperactive TGF- β signals in MPS-I are unknown. The similar mechanisms leading to hyperactive TGF- β signals may exist in the other types of MPS. The findings of TGF- β hyperactivity in the cardiovascular lesions in a patient with MPS-I may lead to a new therapeutic approach. Further studies are warranted to evaluate the effectiveness of the medications that suppress TGF- β signals, such as losartan, in preventing or improving cardiovascular lesions in patients with MPS.

Abbreviations

MPS Mucopolysaccharidosis
TGF- β Transforming growth factor-beta

Communicated by: Maurizio Scarpa

Competing interests: None declared

S. Yano (✉)
Genetics Division, Department of Pediatrics, LAC + USC
Medical Center, University of Southern California, General
Laboratory Building Rm 1 G-24, 1801 Marengo Street,
Los Angeles 90033 CA, USA
e-mail: syano@usc.edu

C. Li
Research Division, Neonatology Division, Department of
Pediatrics, LAC + USC Medical Center, University of Southern
California, Los Angeles, CA, USA

Z. Pavlova
Department of Pathology, Childrens Hospital Los Angeles,
University of Southern California, Los Angeles, CA, USA

Introduction

Mucopolysaccharidoses (MPS) are a group of genetic disorders due to deficiency of lysosomal enzymes resulting in impaired glycosaminoglycan metabolism. The disorders have heterogeneous clinical phenotypes including natural history and symptoms even among patients with the same type of MPS. Systemic gradual accumulation of glycosaminoglycan in the lysosomes typically causes chronic progressive nature and often involves many organ systems, resulting in early death. Cardiovascular lesions in MPS-I have been studied more intensively compared to other types of MPS. Cardiovascular findings in autopsies on patients with MPS-I (including Hurler, Hurler-Scheie, and Scheie variants) revealed approximately 70% of valvular involvement and

approximately 50% of arterial involvement including coronary artery stenosis (Krovetz et al. 1965). Cardiovascular system failure is one of the major causes of death in patients with MPS-I (Krovetz et al. 1965). “Sudden death” has been reported in patients with MPS-I, which is thought to be due to coronary artery disease or arrhythmias due to primary myocardial involvement (Krovetz et al. 1965; Yano et al. 2009). Autopsy specimens from a patient with MPS-I showed enlarged heart with markedly thickened left ventricular walls, thickened aortic and mitral valves, and endocardial fibroelastosis. Microscopic studies showed the findings of hypertrophic cardiac muscle fibers, diffuse increase in fibrous tissues, and stenosis of the major coronary arteries (Yano et al. 2009). The stenotic lesions are mainly due to thickening of the intima. Histopathologic similarity in the coronary artery lesions between the atherosclerotic changes in adults and in MPS-I has been reported (Renteria et al. 1976; Brosius and Roberts 1981).

The mechanisms which cause cardiovascular changes including coronary artery stenosis, endocardial fibroelastosis, thickened valvular lesions, and hypertrophic cardiomyopathy in MPS-I have not been well characterized. Immunohistochemical studies were conducted with the canine MPS-I models and demonstrated increased fibronectin and transforming growth factor beta-1 (TGF- β 1) signaling in the vascular lesions (Lyons et al. 2011). Involvement of over expression of TGF- β 1 signaling in cardiomyopathy and cardiovascular fibrosis has been reviewed (Ruiz-Ortega et al. 2007; Khan and Sheppard 2006).

Immunohistochemical studies were conducted in the cardiac specimens to evaluate transforming growth factor-beta (TGF- β) activities in the coronary arteries, endocardium, and myocardium to find out its involvement in the coronary and cardiac lesions in a patient with MPS-I (Yano et al. 2009).

Methods

This study was approved by the University of Southern California Institutional Review Board (HS-10-00375). Phosphorylated Smad2 (p-Smad2) immunofluorescent staining was performed on cardiac specimens from the patient with MPS-I previously reported (Yano et al. 2009). Formalin-fixed 5 μ m sections were prepared from paraffin-embedded cardiac specimens and mounted on poly-L-lysine-coated slides. The slides were then deparaffinized in xylene and rehydrated. After incubation with primary antibody against p-Smad2 (rabbit polyclonal, Cell Signaling Technology), Cy3-conjugated donkey anti-rabbit secondary antibody (Vector Laboratories) was applied for one hour at room temperature. Sections were preserved in VECTASHELD mounting medium with DAPI (to visualize nuclei).

Results

Phosphorylated Smad2 staining in postmortem cardiac specimens from a patient with MPS-I showed increased activities in the intimal layer with myointimal proliferation as well as in the tunica adventia (Fig. 1a). Figure 1b showed significantly increased activities of phosphorylated Smad2 in the left myocardium. The age matched control showed very few phosphorylated Smad2 signals in the vascular walls as well as in the myocardium (Fig. 1c).

Fig. 1 Phosphorylated Smad2 (p-Smad2) stain. (a) Patient X100, (b) Patient X 200, (c) Control X 100. (a) Extramural coronary artery section showing strong positive p-Smad2 signals (red fluorescence) in the intimal and external vascular wall. Note myointimal proliferation with prominent alpha smooth muscle signals (green fluorescence). (b) Myocardial cells showing strong p-Smad2 signals throughout the section. (c) Section showing myocardial cells and intramural coronary arteries from an age matched control with very few p-Smad2 signals compared to the patient

These findings suggest that TGF- β signal pathway is involved in coronary artery myointimal proliferation causing stenosis and myocardial hypertrophy with fibrotic changes (Fig. 1a, b). Phosphorylated Smad2 signals were increased in the thickened endocardium compared to the age matched control: 40% of cells were positive for phosphorylated Smad2 signals in the patient. Less than 5% were positive in the control (data not shown).

Discussion

There was no specific treatment available for any type of MPS until bone marrow transplantation was first reported in 1981 by Hobbs (Hobbs et al. 1981). Since enzyme replacement therapy (ERT) for MPS-I was introduced in 2003, it has been widely recognized that some clinical symptoms progress despite ERT. Mental retardation, for example, is well recognized and predicted to progress since the therapeutic enzyme can not go through the blood brain barrier. Development of cardiovascular complications including cardiomyopathy and valvular abnormalities are also well known in patients with MPS-I who have been treated with ERT (Sifuentes et al. 2007).

To study the mechanisms causing hypertrophic cardiomyopathy with fibrosis in the left ventricular wall, endocardial fibroelastosis, and the coronary artery lesions with intimal proliferation resulting in stenosis, immunohistochemical studies were performed on the pathology specimens from the patient with MPS-I to evaluate involvement of TGF- β activities. The study showed significantly increased phosphorylated Smad2 signals in the myocardial cells, in the endocardial cells, and in the coronary artery walls in the specimens from the patient with MPS-I. These findings suggest that TGF- β signal pathway is involved in these lesions (Fig. 1a, b). In mammals, TGF- β has three isoforms: TGF- β 1, TGF- β 2, and TGF- β 3. TGF- β 1 is expressed in myofibroblasts, vascular smooth muscle cells, endothelial cells, and macrophages. TGF- β 1 leads to phosphorylation of Smad2 protein through the process of binding to a dimerized receptor, consisting of TGF- β 1 receptor 1 and TGF- β 1 receptor 2, found on the cell surface (Khan and Sheppard 2006). In the heart, it has been postulated that the effects of TGF- β 1 are primarily mediated through Smad2 phosphorylation (Pokharel et al. 2002).

Although the primary cause of TGF- β hyperactivity in the cardiovascular lesions in a patient with MPS-I is unclear, increased TGF- β activity is known to up-regulate beta1,3-glucuronosyltransferase-I in fibroblasts, a rate-limiting enzyme in glycosaminoglycan synthesis, which leads to increase synthesis of glycosaminoglycans including dermatan sulfate, hyaluronic acid, and chondroitin sulfate

A and C (Venkatesan et al. 2011; Matalon and Dorfman 1968). It is also known that accumulated dermatan sulfate can activate STAT proteins which increase productions of elastin-degrading proteins, i.e., matrix metalloproteinase-12 (MMP-12) and cathepsin S. (Ma et al. 2008). Reduction of elastin in myocardial cells, endocardium, and the coronary artery may lead to proliferation of connective tissues, resulting in fibrosis in the ventricular walls, endocardium, and vascular walls (Hinek and Wilson 2000; Karnik et al. 2003).

As reported (Yano et al. 2009), ERT may not be able to prevent or improve the cardiovascular changes. It has become widely known among treating physicians that skeletal lesions and cardiac lesions, particularly valvular lesions, often do not respond to ERT. The observation of TGF- β 1 hyperactivity in the cardiovascular lesions in a patient with MPS-I may lead to a new therapy to prevent the life threatening cardiovascular changes.

In summary, we showed evidence of involvement of TGF- β hyperactivity in the cardiovascular lesions including hypertrophic cardiomyopathy, endocardial fibroelastosis, and the coronary stenosis in a patient with MPS-I. Our findings may suggest that medications which inhibit TGF- β activities, such as losartan, may have beneficial effects in preventing these life threatening complications in patients with MPS-I. Immunohistochemical studies in patients with other types of MPS are also indicated since the similar pathogenesis may exist. Further studies are warranted to evaluate the effectiveness of the TGF- β inhibitors on cardiovascular lesions in patients with MPS.

Synopsis

TGF- β hyperactivity is involved in cardiovascular lesions in MPS-I.

References to Electronic Databases

MPS-I (Hurler disease) MIM 607014
Transforming growth factor-beta (TGF- β)

References

- Brosius FC, Roberts WC (1981) Coronary artery disease in the Hurler syndrome: qualitative and quantitative analysis of the extent of coronary narrowing at necropsy in six children. *Am J Cardiol* 47 (3):649–653
- Hinek A, Wilson SE (2000) Impaired elastogenesis in Hurler disease: dermatan sulfate accumulation linked to deficiency in elastin-binding protein and elastic fiber assembly. *Am J Pathol* 156 (3):925–938
- Hobbs JR, High-Jones K, Barrett AJ et al (1981) Reversal of clinical features of Hurler's disease and biochemical improvement after

- treatment by bone-marrow transplantation. *Lancet* 2 (8249):709–712
- Karnik SK, Brooke BS, Bayes-Genis A et al (2003) A critical role of elastin signaling in vascular morphogenesis and disease. *Development* 130(2):411–423
- Khan R, Sheppard R (2006) Fibrosis in heart disease: understanding the role of transforming growth factor- β 1 in cardiomyopathy, valvular disease and arrhythmia. *Immunology* 118:10–24
- Krovetz LJ, Lorincz AE, Schiebler GL (1965) Cardiovascular manifestations of the Hurler syndrome: hemodynamic and angiocardiographic observations in 15 patients. *Circulation* 31:132–141
- Lyons JA, Dickson P, Wall J et al (2011) Arterial pathology in canine mucopolysaccharidosis-1 and response to therapy. *Lab Invest* 91 (5):665–674
- Ma X, Tittiger M, Knutsen RH et al (2008) Upregulation of elastase proteins results in aortic dilation in mucopolysaccharidosis I mice. *Mol Genet Metab* 94(3):298–304
- Matalon R, Dorfman A (1968) The structure of acid mucopolysaccharides produced by hurler fibroblasts in tissue culture. *Proc Natl Acad Sci USA* 60(1):179–185
- Pokharel S, Rasoul S, Roks AJM et al (2002) N-acetyl-Ser-Asp-Lys-Pro inhibits phosphorylation of Smad2 in cardiac fibroblasts. *Hypertension* 40:155–161
- Renteria VG, Ferrans VJ, Roberts WC (1976) The heart in the Hurler syndrome: gross, histologic and ultrastructural observations in five necropsy cases. *Am J Cardiol* 38(4):487–501
- Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J (2007) TGF- β signaling in vascular fibrosis. *Cardiovasc Res* 74:196–206
- Sifuentes M, Doroshow R, Hofst R et al (2007) A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years. *Mol Genet Metab* 90(2):171–180
- Venkatesan N, Ouzzine M, Kolb M, Netter P, Ludwig MS (2011) Increased deposition of chondroitin/dermatan sulfate glycosaminoglycan and upregulation of β 1,3-glucuronosyltransferase I in pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 300(2):L191–L203
- Yano S, Moseley K, Pavlova Z (2009) Postmortem studies on a patient with mucopolysaccharidosis type I: histopathological findings after one year of enzyme replacement therapy. *J Inher Metab Dis*. doi 10.1007/s10545-009-1057-4