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Expression of bone morphogenetic protein 4 and its receptors in the remodeling heart

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ABSTRACT

Aims: Heart failure is associated with activation of fetal gene programs. Bone morphogenetic proteins (BMPs) regulate embryonic development through interaction with BMP receptors (BMPRs) on the cell surface. We investigated if the expression of BMP4 and its receptors BMPR1a and BMPR2 were activated in post-infarction remodeling and heart failure.
Main methods: Left ventricular biopsies were taken from explanted hearts of patients with end-stage heart failure due to dilated cardiomyopathy (CMP; n = 15) or ischemic heart disease (CAD; n = 9), and compared with homograft control preparations from organ donors deceased due to non-cardiac causes (n = 7). Other samples were taken from patients undergoing coronary artery bypass grafting (CABG; n = 11). Mice were subjected to induced infarction by permanent coronary artery ligation or sham operation, and hearts were sampled serially thereafter (n = 7 at each time point).
Key findings: Human and mouse hearts expressed BMP4 and both receptor subtypes. CABG and CMP patients had increased expression of mRNA encoding for BMP4, but unchanged protein. Mouse hearts had increased BMP4 precursor protein 24 h after infarction. BMPR1a protein decreased in CAD patients and initially in postinfarcted mouse hearts, but increased again in the latter after two weeks. Human recombinant BMP4 promoted survival after H_2O_2 injury in HL-1 cells, and also protected adult mouse cardiomyocytes against hypoxia–reoxygenation injury.
Significance: Adult hearts express BMP4, the mRNA increasingly so in patients with coronary artery disease with good cardiac function. BMPRs are downregulated in cardiac remodeling and failure. Recombinant BMP4 has protective effects on cultured cardiomyocytes.

Introduction

Bone morphogenetic proteins (BMPs) are growth factors in the transforming growth factor β (TGFβ) superfamily. The more than 20 members of BMPs can be subdivided into six groups based on their structure and function (van Wijk et al., 2007; Ehrlich et al., 2011). They are produced as inactive precursor proteins which are activated by endoproteolytic cleavage (van Wijk et al., 2007). BMP4 binds either to a type 1 or type 2 receptor to form the ternary signaling complex. The type 2 receptor activates the type 1 receptor by phosphorylation. The latter activates small mothers against decapentaplegic 1, 5 or 8, which migrate to the nucleus and stimulate BMP-specific target genes, such as the cell differentiation regulator DNA-binding inhibitor protein 1 (ID-1) (Chang et al., 2002). ID-1 has effects on cell growth, senescence, and differentiation (Chang et al., 2002). Detailed descriptions of the signaling pathway were recently reviewed (Euler-Taimor and Heger, 2006). BMPs are involved in numerous cellular functions during development and adult life. These include formation of cartilage and bone, differentiation of the vertebrate nervous system, and mediating apoptosis during embryogenesis. BMPs have key functions in embryonic development of the heart (Xiao et al., 2007).

Heart failure is a functional consequence of a wide variety of diseases, including ischemic heart disease and pressure overload (Chung et al., 2003; Francis and Tang, 2003). In response to chronically increased workload or wall stress, myocardial tissue undergoes a series of cellular and molecular alterations to adapt to the increased demands (Chung et al., 2003). This process is known as cardiac remodeling, and is

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manifested by changes of myocardial tissue structure, chamber geometry and cardiac function (Pachori et al., 2010). Activation of fetal gene programs appears to be a central molecular event in cardiac remodeling (Chung et al., 2003). The fetal transcription factors such as NFAT, MEF2, GATA4-6 and NK-2 activate a multitude of genes, including beta-myosin heavy chain, Myc, JunD, connective tissue growth factor and others, which may contribute to heart failure development (Kinugawa et al., 2001; Ricci et al., 2005; Chen et al., 2013; Dirkx et al., 2013).

BMP4, BMP receptor and SMAD knockout mouse models have distinct phenotypes summarized elsewhere (Chang et al., 2002). The phenotypes include embryonic death due to non-development of the heart at different stages depending on the gene or receptor which is deleted (van Wijk et al., 2007). BMP4 has been implicated in the development of endocardial cushion (van Wijk et al., 2007). Mice lacking the BMP4 gene have gross alterations of heart formation (McCulley et al., 2008). A conditional knock-out of BMPR1a/Alk3 in heart progenitor cells resulted in a failed progression of the first heart field (Klaus et al., 2007), while conditional deletion of BMPR1a hampers development of the atrioventricular conduction system (Stroud et al., 2007).

As activation of fetal gene programs is a feature of heart failure, we hypothesized that BMP4 and its receptors were activated in failing human and/or remodeling mice hearts. BMP4 function was investigated in mouse heart cells.

Materials and methods

Biopsies of human hearts

The study conforms with the ethical standards laid down in the 1964 Declaration of Helsinki, and it was accepted by the Regional Ethics Committee (Regional Komite for Medisinsk og Helsefaglig Forskningsetikk Sør-Øst, REK 07482a). Informed, written consent was obtained from all patients or their families. Samples from the left ventricular free wall were removed from still-beating hearts immediately on explantation from patients with end-stage heart failure undergoing cardiac transplantation. Fifteen biopsies from patients with dilated cardiomyopathy (CMP) and 9 from patients with heart failure due to ischemic heart disease (CAD) (NYHA class IV) were included. Tissues were snap-frozen in liquid nitrogen and kept at −80 °C. Control human left ventricular tissue was obtained from 7 sex- and age-matched subjects whose valves were used for homograft preparations. None of the donors had a history of heart disease. Their myocardium was kept on ice for 1 to 4 h before biopsies were taken. A group of 11 patients with coronary artery disease without heart failure, but scheduled for elective coronary artery bypass grafting (CABG) (NYHA class 2.8 ± 0.6, mean ± SD), were included in the study. From this group, fine needle biopsies were sampled from the free wall of the left ventricle before the start of extracorporeal circulation, and immersed in liquid nitrogen in the operating theater. The size of the sample was sufficient for RNA analysis through real-time PCR, but not for protein analysis. Patient characteristics are shown in Table 1.

Table 1
Characteristics of the patient material. Left ventricular biopsies were sampled from patients with heart failure due to dilated cardiomyopathy (CMP), or coronary artery disease (CAD) undergoing heart transplantation. Patients with coronary artery disease undergoing elective coronary artery bypass grafting (CABG) were included. They were compared with biopsies of the hearts from donors used for homograft preparations (no clinical data available). Ejection fraction (EF), cardiac output (CO), cardiac index (CI), and number of patients with preoperative myocardial infarctions (preopMI) are shown as mean ± SD.

Group	Age	F/M	EF	CO	CI	preopMI
CMP, n = 15	40 ± 18	3/12	21.9 ± 7.5	4.1 ± 1.7	2.1 ± 0.7	0
CAD, n = 9	55 ± 14	0/9	23.1 ± 6.2	4.9 ± 1.7	2.3 ± 0.6	9
CABG, n = 11	68 ± 8	3/8	>60	n.m.	2.8 ± 0.5	4

n.m. = not measured. M = male, F = female.

Animals

Male C57BL/6 mice (25–30 g) were used, and handled according to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health. The study was approved by the local ethics committee for animal research (Forsøksdyrutvalget Avdeling for Komparativ Medisin IMB, ID 1092).

Study design

Male C57BL/6 mice (25–30 g) were subjected to coronary artery ligation or sham treatment (n = 7 in each group at all time points). Hearts were harvested 24 h later, and 2 and 4 weeks later for real time PCR and 24 hour, 1, 2, 4 and 6 week samples were analyzed with Western blot. Additional hearts were sampled from untreated 2 days old (P2, n = 7) and adult mice (n = 7). The phenotype of cardiac remodeling was confirmed using transthoracic ultrasound performed with the Vevo 770 system using a 13-MHz linear array transducer preoperatively and 6 weeks after surgery. Two dimensional guided M-mode recordings in sagittal and frontal axes were obtained; the derived functional parameter of ejection fraction was calculated. All dimensions were analyzed using the EchoPac software analysis program (GE Vingmed Ultrasound, Horten, Norway). Data are presented in Supplementary Fig. 1. The surgical technique was the same for all animals as described earlier with minor modifications (Michael et al., 1995). Mice were anesthetized with isoflurane, intubated and immobilized on a heating pad. They were ventilated at a respiratory rate of 135/min with pure oxygen mixed with 1.5–2.0% isoflurane. After skin incision, the left pectoral muscles were prepared free and retracted, and the intrathoracic cavity opened through the third or fourth intercostal space. After exposing the heart, the left coronary artery was ligated 1.5 mm under the tip of the left auricle using a 6/0 silk suture to ensure similar infarct sizes. Sham treatment consisted of thoracotomy and a silk suture placed around the coronary artery, but without subsequent ligation. The intercostal space and the skin were closed using 5/0 polyester suture. Animals were kept at 30 °C overnight, receiving 0.5 ml saline intraperitoneally for fluid replacement and 0.5 mg/kg buprenorphine subcutaneously for analgesia. Administration of buprenorphine was repeated when animals presented signs of pain.

Table 2
Human and murine primer sequences used in the present study. SYBR Green was used for detection. Rpl32 was used as endogenous control in HL-1 cells, while predesigned primers and probes were used as endogenous control for human and murine heart biopsies (HPRT and 18s, respectively).

Gene		Sequence
Human		
BMPR2	3′	GCTGTTGTAGCACAGATTTATGTAATGT
	5′	GATGCCAAAGCAATGATTATTGTC
Alk3 (BMPR1a)	3′	AACCAGTATTTGCAACCCACACT
	5′	AGCAAAACCAGCCATCGAAT
BMP2	3′	GCAGCTTCCACCATGAAGAATC
	5′	GAAGCTCTGCTGAGGTGATAAACTC
BMP4	3′	ACCACGAAGAACATCTGGAGAAC
	5′	GGGATGCTGCTGAGGTTAAAGA
Mouse		
BMPR2	3′	TCTTCCCAGGTGAATCTGTACCA
	5′	GGGAACTTGGGTCTCTGCTTCT
Alk3 (BMPR1a)	3′	ATTTCTCATGTTCAAGGGCAGAA
	5′	GCAGTGTCCTGAGCAATAGCACTTTA
BMP4	3′	GCCACCATGATTCCTGGTAACCGAATGCTGATG
	5′	TCACGCGCATAAACACCCCT
BMP2	3′	GCCGGCCTCATTCCAGA
	5′	TGAGCAGCCTCAACTCAAATTC
rpl32	3′	TCGTCAAAAAGAGGACCAAGAAG
	5′	CCGCCAGTTTCGCTTAATTT