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Title: Possible target for preventing fibrotic scar formation following acute myocardial infarction

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INTRODUCTION

Acute myocardial infarction

Acute myocardial infarction (AMI) is the leading cause of death in developed countries and presents a major public health problem (1). It mainly occurs after coronary artery occlusion as a result of loss of myocardial tissue blood supply (2). Myocardial tissue that no longer receives adequate blood dies rapidly and is replaced with poorly functioning or non-functional fibrotic scar tissue which can expand leading to increased loss of functional myocardial tissue, which in turn can result in a dysfunctional heart (3). More than one-half of a million people experience a first AMI each year in the United States, and over two hundred thousand people suffering from myocardial infarction die before reaching a hospital.

Treatments for AMI are typically effective only if implemented rapidly after occlusion of the coronary vessel (4). Aggressive thrombolytic therapies include drugs that dissolve thrombi or primary angioplasty and stents (5). Chronic, post-infarction treatments include angiotensin-converting enzyme inhibitors, beta blockers, diuretics and calcium channel antagonists, which can reduce aortic pressure; thereby decreasing ventricular remodeling of the left ventricle (LV) that otherwise can expand the size of the infarct leading to more non-functional scar tissue. Open-heart surgical methods include coronary artery bypass surgery to repair or replace occluded coronary vessels and methods to repair, shrink, or remove the non-functional infarcted region of heart tissue.

BMP1 isoforms

Bone morphogenetic protein 1 (BMP1) was originally isolated from highly purified

BMP bovine bone extracts and was reported to induce formation of cartilage *in vivo* in a subcutaneous bone formation assay (6). However, BMP1 does not share significant amino acid sequence homology with other BMPs, which are members of the TGFβ super family of growth factors. BMP1 was shown to be identical to procollagen C-proteinase, which is a zinc metalloproteinase that cleaves the carboxyl pro-domains of procollagens I, II and III to produce mature monomers of the major fibrillar collagens I, II and III (7).

This event is essential for the proper assembly of insoluble collagen within the extracellular matrix (ECM) and the formation of scar tissue as found associated with a variety of organ disorders. In addition to its role in cleaving procollagen, BMP1 cleaves other ECM macromolecules, including prolysyl oxidase (8), probiglycan (9) and prolaminin-5 (10). The BMP1 is now known to be an essential control point of morphogenesis during the cascade of pattern formation (11). Mice null for the BMP1 gene are perinatal lethal with failure of ventral body wall closure and persistent gut herniation, probably due to defective ECM and limited disruption of dorsoventral patterning (12). Consistent with a loss of pCP activity, BMP1-null mice have abnormal collagen fibrils. BMP1/TLD-related metalloproteinases are responsible for the proteolytic maturation of a numerous of extracellular proteins related to formation of the ECM. These include various collagens, small leucine-rich proteoglycans, lysyl oxidase and laminin-5 (13). The originally discovered form of BMP1 is designated as "BMP1-1" and other BMP1 isoforms encoded by splice variant RNA transcripts have been described on the transcriptional level and designated with sequential suffixes: BMP1-2, BMP1-3, BMP1-4, BMP1-5, BMP1-6, and BMP1-7 (14). As expected, the BMP1 isoforms encoded by the splice variant transcripts share a number of domains, including leader peptide, proregion, and protease (catalytic) region. Recently, a

number of BMP1 isoforms have been confirmed at the protein level as circulating in the blood of patients with various diseases, such as chronic kidney disease and acute pancreatitis, and in the blood of healthy human individuals, which only contains BMP1-3 (15). Moreover, the role of BMP1-3 in processing procollagen and possibly leading to fibrosis and scar tissue in a variety of diseases is an attractive target for developing new therapies.

HYPOTHESIS

The intricate relationships among the cellular and acellular components of the heart drive the proper heart development, homeostasis and recovery following pathological injury. Cellular myocytes, fibroblasts and endothelial cells differentially express and respond to particular extracellular matrix factors that contribute to cell communication and overall cardiac function. ECM facilitates mechanical, electrical and chemical signals during homeostasis and the developmental process. These signals modulate cellular activities such as cell proliferation, migration, adhesion and changes in the gene expression. During various physiological cardiac states different cellular and ECM expression changes take place (16). For example, during myocardial infarction myocytes undergo apoptosis, fibroblasts undergo intensive proliferation, vascular density decreases and an increased expression of collagen I, III, IV, fibronectin and periostin leads to enhanced fibrosis and diminished cardiac function. These processes have adverse effects on left ventricular function, thus forming a therapeutic basis for use of anti-fibrotic agents to inhibit or reverse such adverse effects (17).

Fibrosis diseases account for up to 45% of deaths in the developed world, yet there are no approved antifibrotic therapies (18). Translating advances in experimental fibrosis to clinical use has been challenging in the past, because there are now many candidate antifibrotic targets, and most are tested usually only in a single organ type and species. The discovery of circulating organ specific BMP1 isoforms represents a ground-breaking finding in the field of fibrosis-related human diseases. Exploration of their structure/function relationship and characterization of their potential for processing target substrates, as well as their role in the regulation of the activity of growth factors, specifically morphogens from the TGFβ superfamily, could be of

major medical importance.

BMP1-3 in the circulation might have multiple, yet unpredicted functions in orchestrating the ECM assembly globally and locally in regulating latent growth factors activity affecting the pathophysiology of major human diseases with fibrosis as a common outcome. BMP1 is involved in the biosynthetic processing of a range of ECM precursors and releases several TGFβ superfamily members, including BMP2 and BMP4, growth and differentiation factors GDF 8/11, and TGFβ1 from their latent complexes or antagonists. These dual roles have fuelled speculation that BMP1 proteinases orchestrate ECM assembly by signaling through TGFβ-like proteins.

Today, conventional therapy for AMI are not specific for cardiac fibrosis, but are aimed to improve heart function, mainly by preventing/reducing ventricular remodeling (ACE inhibitors, aldosterone antagonists, β blockers) or preventing/reducing progression of coronary artery disease (antiplatelet drugs and statins). Despite the fact that there are 544 currently ongoing cardiovascular clinical trials, out of which 342 are related to drugs and 157 to various drug targets, there are no effective and safe drugs or therapies to treat fibrosis.

Therefore, we hypothesize that BMP1-3 represents a core pathway protein essential in fibrotic events following acute myocardial infarction and its targeting may be sufficient to limit fibrosis progression. Moreover, administration of an monoclonal antibody to BMP1-3 could be effective in decreasing the extent of myocardial tissue damage and even to promote regeneration of functional myocardial tissue in the infarcted region. Furthermore validation of our novel target molecules and the development of neutralizing antibodies as therapeutic options to prevent fibrosis after AMI would represent a major step forward.

CONSEQUENCES OF THE HYPOTHESIS AND DISCUSSION

Until recently, regeneration of the heart was thought to be restricted to fish and amphibians which retain a robust capacity for cardiac regeneration throughout life. It has been shown in zebrafish that genetic regulatory hierarchy of developing myocardium involves a close relationship between cardiac precursors and specific signaling molecules, including GATA, BMP and Nkx (19). Hearts of zebrafish can regenerate completely by proliferation and differentiation of mature cardiac myocytes within two months of surgical amputation of up to 20% of the ventricle (20, 21), but this is not the same in the adult mammals. Recently, it has been found that the hearts of neonatal mice can regenerate by cardiomyocyte proliferation after partial surgical resection, but this capacity is lost by seven days of age (22). Several studies have shown evidence for cardiac stem cell populations within the postnatal mammalian heart, likely to form cells with cardiac myocyte properties in culture and after transplantation back into the heart (23). At least one of these progenitor populations contributing to endogenous repair are the cells of the epicardium (24). Although it is unlikely to expect that regeneration of cardiac tissue in humans will be as effective as in amphibians and fish, prevention of scar formation and control of its degradation may now be a realistic option. We hypothesize that inhibition of BMP1-3, when given to rats with AMI, influences the scar size and composition eventually preventing an excessive scar formation. It is also not known whether the scar composed of less collagens I-III and more other ECM will make it prone to future reverse remodeling and/or replacing fibrous with regenerated muscle tissue. Interestingly, in line with this, amphibians and fish following heart injury and limb amputation do not form scars, but cover the injured surface by a layer of epithelial cells and little extracellular

matrix which is not composed of cross-linked collagens. Therefore, the role of BMP1-3 in heart repair following a heart injury model in zebrafish will also be interesting issue.

Recently was shown that current approaches to drug target discovery are not fully successful in identifying relevant and specific targets (25). Our hypothesis suggests a different point, utilizing proteomic analysis and exploring target proteins with already proven preliminary in vivo efficacy. Based on our preliminary findings we will characterize, validate and test BMP1 isoforms, as new targets for AMI and develop neutralizing antibodies as new therapeutic tools.

The advancement of the current knowledge will be primarily achieved by gathering information about BMP1 isoforms molecular structure and biochemical properties. For that purpose it will be necessary to produce labelled recombinant protein isoforms and altered protein forms to characterize the specific amino acids critical for substrate binding. This knowledge will be utilized for engineering specific therapeutic antibodies against individual BMP1 isoforms. Additionally, applying proteomic and genomic approaches to generate the expression profiles of organ proteins in the course of myocardial infarction, it will be able to identify BMP1 isoform extra cellular targets in animal experiments using the existing and newly made specific acute myocardial infarction models. These newly identified proteins will present the “first line” target molecules for exploring the BMP1 isoform specific substrate candidates and the mechanism of action.

If this step will be successful, it will be able to construct diagnostic tools based on protein probes for the BMP1 isoforms. Since fibrogenesis is both a systemic and a local disease, each organ has a specific pathophysiology of fibrosis and it is unlikely that one molecule might prevent the progress of fibrosis. Therefore, a better

understanding of the role of individual BMP1 isoform – BMP1-3 will enable to test it in preventing the disease progress. This new, risky and innovative approach should be applied in treating myocardial fibrotic conditions.

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