Wnt-induced secreted protein-1 is a prohypertrophic and profibrotic growth factor

J. T. Colston,1 S. D. de la Rosa,1 M. Koehler,1 K. Gonzales,1 R. Mestril,2 G. L. Freeman,1,3 S. R. Bailey,1 and B. Chandrasekar1,3

1Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas; 2Department of Physiology and Cardiovascular Institute, Loyola University Medical Center, Maywood, Illinois; and 3South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, Texas

Submitted 6 April 2007; accepted in final form 4 July 2007

Colston JT, de la Rosa SD, Koehler M, Gonzales K, Mestril R, Freeman GL, Bailey SR, Chandrasekar B. Wnt-induced secreted protein-1 is a prohypertrophic and profibrotic growth factor. Am J Physiol Heart Circ Physiol 293: H1839–H1846, 2007. First published July 6, 2007; doi:10.1152/ajpheart.00428.2007. —Wnt-1-induced secreted protein-1 (WISP-1) is a member of the cysteine-rich 61, connective tissue growth factor, and nephroblastoma overexpressed (CCN) family of growth factors and is expressed in the heart at low basal levels. The purpose of this study was to investigate whether WISP-1 is upregulated in postinfarct myocardium and whether WISP-1 exerts prohypertrophic and mitogenic effects stimulating myocyte hypertrophy, cardiac fibroblast (CF) proliferation, and collagen expression. Male C57Bl/6 (25 g) mice underwent permanent occlusion of the left anterior descending coronary artery. mRNA and protein levels were analyzed by Northern and Western blot analyses. Cardiomyocyte hypertrophy was quantified by protein and DNA synthesis. CF proliferation was quantified by CyQuant assay, and soluble collagen release by Sircol assay. A time-dependent increase in WISP-1 expression was detected in vivo in the noninfarct zone of the heart. WISP-1 expression preceded WISP-1 expression in vivo and stimulated WISP-1 expression in neonatal rat ventricular myocytes in vitro. WISP-1-induced cardiomyocyte hypertrophy was evidenced by increased protein (2.78-fold), but not DNA synthesis, and enhanced Akt phosphorylation and activity. Treatment of primary CF with WISP-1 significantly stimulated proliferation at 48 h (6.966 ± 264 vs. 5.476 ± 307 cells/well, P < 0.01) and enhanced collagen release by 72 h (18.4 ± 3.1 vs. 8.4 ± 1.0 ng/cell, P < 0.01). Our results demonstrate for the first time that WISP-1 and biglycan are upregulated in the noninfarcted myocardium in vivo, suggesting a positive amplification of WISP-1 signaling. WISP-1 stimulates cardiomyocyte hypertrophy, fibroblast proliferation, and ECM expression in vitro. These results suggest that WISP-1 may play a critical role in post-myocardial infarction remodeling.

WNT1-INDUCED secreted protein-1 (WISP-1) is a member of the cysteine-rich 61, connective tissue growth factor (CTGF), and nephroblastoma overexpressed (CCN) family of growth factors shown to play a role in cellular growth, transformation, and survival (24, 26, 52, 57). Because of the limited regenerative capacity of the heart, any structural adaptations of the myocardium occurring in response to chronic changes in hemodynamic loading conditions typically require alterations in the size of myocytes and composition of the interstitial components (46). When the stimulus is chronic pressure overload, as in hypertension or aortic stenosis, cardiac myocytes increase in diameter and there is increased deposition of interstitial collagen (27). In contrast, chronic volume overload, as occurs with valvular regurgitation, leads to lengthening of myocytes with less exuberant deposition of interstitial collagen (49). In the unique setting of myocardial infarction (MI) the process is more complicated, since the necrotic tissue must undergo healing with formation of a sturdy fibrotic scar, whereas the remaining noninfarcted tissue must perform increased hemodynamic work (45). This process leads to myocyte hypertrophy, fibroblast proliferation, and enhanced collagen deposition in the noninfarcted zone of the heart.

The CCN family of growth factors include cysteine-rich 61, CTGF, nephroblastoma overexpressed, WISP-1, WISP-2, and WISP-3. The cellular targets of WISP-1 and other CCN proteins are specific and include myocytes, fibroblasts, endothelial cells, and certain tumor cells (4, 24, 26, 29). The CCN proteins have been shown to exert potent and overlapping effects on cell growth, proliferation, and extracellular matrix (ECM) expression. In particular, CTGF expression is induced by transforming growth factor-β (TGF-β) in cardiac myocytes and fibroblasts and has been shown to play a role in interstitial remodeling (46). Since WISP-1 is expressed in the heart and cardiac fibroblast (CF) proliferation and ECM expression are central to cardiac remodeling, it was hypothesized that WISP-1 plays a role in the remodeling process.

The purpose of this study was to investigate the expression and induction of WISP-1 in postinfarcted myocardium in vivo and to explore the effects of WISP-1 on cardiomyocytes and CFs in vitro. Our results demonstrate for the first time that WISP-1 is expressed in vivo in the noninfarcted myocardium following permanent left anterior descending coronary artery (LAD) occlusion and stimulates cardiomyocyte hypertrophy, fibroblast proliferation, and ECM expression in vitro. These results suggest that WISP-1 may play a critical role in post-MI remodeling.

MATERIALS AND METHODS

Animals and MI. The investigation conforms with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health, and all protocols were approved by the Institutional Animal Care and Use Committee. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tional Animal Care and Use Committee of the University of Texas Science Center at San Antonio (41a). MJ studies were performed in male C57Bl/6 mice (n = 4/group) byligating the LAD using the closed-chest mouse model described by Entman and colleagues (42, 43). In brief, mice weighing 25–30 g and aged 3 to 4 mo were anesthetized by urethane (1 g/kg ip) and etomidate (25 mg/kg ip) and mechanically ventilated with a rodent ventilator set at 150 breaths/min (100% oxygen). Mice were placed on a heated, temperature-controlled operating table for small animals (Vestavita Scientific). Dissection was aided by a microscope (Surgical Microscope System, Applied Fiberoptics), and the chest was opened along the left side of the sternum by cutting through the ribs to approximately the midsternum. The chest walls were retracted using 6-0 suture. The pericardium was then gently dissected to allow visualization of coronary artery anatomy. An 8-0 Surgipro monofilament polypropylene (PE) suture with the U-shaped tapered needle was passed under the LAD. The needle was then cut from the suture, and the two ends of the 8-0 suture were then thread ed through a 0.5-mm piece of PE-10 tubing that was previously soaked in 100% alcohol overnight, forming a loose snare around the LAD. Each end of the suture was then exteriorized through each side of the chest wall. The chest was then closed with four interrupted stitches using 6-0 sutures. The ends of the exteriorized 8-0 suture were then tucked under the skin, which was then also closed with 6-0 sutures. At 1 wk after instrumentation, the animals were reanesthetized and randomly assigned to sham-operated or MJ groups. The 8-0 suture, which had been previously exteriorized outside the chest wall, was cleared of all debris from the skin and chest and carefully taped to heavy metal picks. Ischémia was accomplished by gently pulling the heavy metal picks apart until an S-T elevation appeared on the ECG (see Fig. 1A). Sham-operated animals (n = 4/group) were prepared identically without undergoing the ischemia protocol. At the end of the experimental period, mice were placed on a small animal respirator, and the chests were opened allowing visual inspection of the beating hearts. The infarcted zone of the left ventricle was visually identified by marked thinning and bulging (9). Careful note was made of anatomic landmarks of this region. The hearts were then rapidly excised and rinsed in ice-cold physiological saline. The right ventricle and atria were trimmed away, and the left ventricle was divided into ischemic and nonischemic zones and snap frozen in liquid N2 for not more than 3 days. All assays were performed in the nonischemic tissue.

Analysis of RNA expression. Gene expression was analyzed by Northern blot analysis as previously described (6). Twenty micrograms of total RNA per lane were denatured, transferred onto nitrocellulose membrane, UV fixed, and probed with [32P]-labeled cDNA. cDNAs were amplified by RT-PCR (IL-1β, GenBank accession No. NM_008361.2; sense, 5'-ATGGCAACTGTTCCTGAACTCAACT-3', antisense, 5'-CAGGACAGTTAGATATCTTTC CTIT-3'; TNF-α, GenBank accession No. NM_013693.1; sense, 5'-GAGTGAACAAAGCTGTAG CCCATGTGTGAGCA-3', antisense, 5'-CCAAGAGTACCTGCC CGCAGT-3'; WISP-1, GenBank accession No. NM_080383.1, sense, 5'-CTCAACAGCTGGGGGACAC-3', antisense, 5'-GATGCCCTCGGCG TGGTACAC-3'; and biglycan, GenBank accession No. NM_011708.1; sense, 5'-TTCACCTGATACCTACGT-3', antisense, 5'-GT TCAAGGCCTGGTTCCTCAG-3') and subcloned into pCR 2.1-TOPO (Invitrogen), and the sequence was confirmed by restriction enzyme analysis and DNA sequencing. To obtain sufficient amounts of cDNA for labeling, the vectors containing specific cDNAs were digested with EcoRI, gel purified, labeled, and used in probing the membranes. 28S rRNA served as an internal control. Autoradiographic bands were semiquantified by videoimage analysis and represent means ± SE of arbitrary numbers obtained (C). MI, myocardial infarction; C, naive. *P < 0.05 and **P < 0.001 vs. respective sham.

Western blot analysis, autoradiography, and densitometry were performed as described previously (8, 10). Briefly, myocardial tissue was homogenized in a hypotonic lysis buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, 1 mM Na3VO4, 10 mM sodium pyrophosphate, 10 mM β-glycerophosphate, 10 mM NaF, and 1% protease inhibitor cocktail (Sigma, St. Louis, MO). Protein concentrations were determined using the BCA assay (Pierce, Rockford, IL) and boiled in SDS sample buffer. Samples were then loaded onto hand-cast 10% polyacrylamide gels using a Bio-Rad Mini Protein System. After electrophoresis, proteins were transferred on to polyvinylidene difluoride membrane and probed with primary antibodies diluted in 2% nonfat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TTBS). After incubation overnight at 4°C, blots were rinsed in TTBS for 30 min and incubated in horseradish peroxidase-conjugated secondary antibodies in 5% milk in TTBS for 1 h. Following a 30-min rinse in TTBS, blots were incubated in chemiluminescent substrate (SuperSignal Pico West; Pierce) supplemented with 5% SuperSignal Femto (Pierce) and exposed to film. Anti-WISP-1 antibodies (AF1680) were purchased from R&D Systems (Minneapolis, MN). Akt (No. 9272) and phospho-Akt (No. 9275) antibodies were from Cell Signaling Technology (Danvers, MA). Akt kinase activity was analyzed using a commercially available kit (Cell Signaling Technology). The assay is based on Akt-induced phosphorylation of glycogen synthase kinase-3 (GSK-3).

Neonatal rat ventricular myocytes. To verify whether WISP-1 exerts prohypertrophic effects, we employed primary neonatal rat ventricular myocytes (NRVMs). Cardiomyocytes were isolated as described previously from 1- to 2-day-old Sprague-Dawley rat hearts by enzyme digestion (11). Human-recombinant WISP-1 (catalog No. 120-18) was purchased from PeproTech (Rocky Hill, NJ). The recombinant protein contained <0.1 ng endotoxin per microgram of...
protein (manufacturer’s data sheet). A 5.26 μm stock was prepared in 10 mM acetic acid, and a working solution was made by diluting the stock in the culture media. Acetic acid (0.038 mM) was prepared as a control, and human WISP-1 has been previously shown to induce DNA synthesis in normal rat kidney fibroblasts (NRK-49F cells) (57). To verify whether WISP-1 mediates hypertrophy of NRVM via activation of Akt, NRVM were treated with α-3-deoxy-2-O-methyl-myo-inositol 1-[β-(2)-methoxy-3-(octadecyloxy)propyl hydroxyl phosphate] (SH-5), an Akt inhibitor II, which was purchased from EMD Biosciences (San Diego, CA). SH-5 functions as a phosphatidylinositol analog and blocks binding of phosphatidylinositol-3,4,5-trisphosphate to the PH domain of Akt (37). SH-5 has been used both in vivo (21) and in vitro (36) to selectively inhibit Akt. We have previously used SH-5 to inhibit IL-18-mediated Akt-dependent NF-κB and activator protein-1 activation in human coronary artery smooth muscle cells (12). Cells were pretreated with 1 μM SH-5 in DMSO for 1 h. At this concentration, SH-5 inhibited Akt activation and did not affect cell survival. DMSO served as a control. Akt activation was also targeted by adeno viral (Ad) vector expressing dominant-negative (dn)Akt. Ad-green fluorescent protein (GFP) served as a control. Cells were infected at 100 multiplicity of infection (MOI) as previously described (13).

Mouse fibroblast isolation and culture. CFs were isolated from the hearts of 8–10-week-old male C57Bl/6 mice using a method of methods developed in our laboratory (16–18). Briefly, after induction of deep anesthesia with an injection (0.1 ml im) of ketamine-acepromazine-xylazine (9:3:1), hearts were rapidly removed, rinsed, and mounted via the aorta onto a 27-gauge cannula attached to a Langendorff-type apparatus allowing retrograde perfusion of the coronary arteries. Hearts were perfused for 5 min with 37°C sterile-filtered calcium-free Krebs-Ringer bicarbonate buffer (KRB) containing (in mM) 110 NaCl, 2.6 KCl, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, and 11 glucose at 80 mmHg. Hearts were then perfused for 20–25 min with KRB-enzyme solution containing 0.5 mg/ml type II collagenase (Worthington Biochemical, Freehold, NJ), 2.5 mM CaCl2, and 1 mg/ml fatty acid-free albumin. After digestion, the ventricles were trimmed free and minced in KRB enzyme solution containing 10 mg/ml albumin, filtered through sterile nylon mesh, and centrifuged at 25 × g for 5 min to remove cardiomycocytes, red blood cells, and debris. The resultant supernatant was then centrifuged at 1,000 g for 8 min. The cell pellet was resuspended in 5 ml CF medium containing 15 mM HEPES, 16.7 mM NaHCO3, basal medium Eagle-vitamins, and MEM-amino acids (1× each; Gibco, Grand Island, NY), 2 mM glutamine, 10% heat-inactivated FBS, and antibiotics (pH 7.3) and plated into T25 tissue-culture flasks (Falcon, Becton-Dickinson Labware, Franklin Lakes, NJ). Nonadherent cells were removed by aspiration after 4 h and discarded. The CFs from 10 mice were cultured until confluent, pooled, plated into T175 flasks, and fed with fresh medium. On reaching confluency, the pooled cells were then used for experiments. CF and nonfibroblast contaminants were identified by immunohistochemical screening as described previously (16–18).

Cytokine-induced WISP-1 expression following MI. Cytokine-induced WISP-1 expression was analyzed in NRVM treated with recombinant-murine (rm)IL-1β, rmTNF-α, or rmIL-1β + rmTNF-α for up to 48 h. The specificity of IL-1β and TNF-α was verified by preincubating NRVM with anti-IL-1β (5 μg/ml, AF-501-NA), anti-TNF-α (5 μg/ml, AF-510-NA), or anti-IL-1β + anti-TNF-α neutralizing antibodies (5 μg each/ml) for 2 h before the addition of respective cytokines. Normal goat IgG (preimmune, 5 μg/ml; R&D Systems) served as a control. WISP-1 expression was analyzed by Western blot analysis in whole cell homogenates.

Total protein synthesis and DNA levels. Cardiomyocyte hypertrophy is characterized by increased protein synthesis without affecting total DNA levels (11). The rate of protein synthesis was determined by measuring the incorporation of [3H]leucine. Briefly, NRVM were plated in 24-well plates, and after overnight culture, the complete medium was replaced with media containing 0.5% BSA. Twenty-four hours later, cells were treated with WISP-1, Forty-two hours later, 0.5 μCi of [3H]leucine was added to the culture medium, and the incubation was continued for an additional 6 h. The radioactivity incorporated into the trichloroacetic acid-precipitable material was determined by using a liquid scintillation counter. Total DNA levels were analyzed in duplicate samples using the DNeasy tissue kit (Qiagen, Valencia, CA). The [3H]leucine incorporation was normalized to DNA, and the ratio of [3H]leucine incorporation/DNA from untreated cells was considered 1, and the results are expressed as fold increase from untreated controls. To investigate the role of Akt, cells were treated with SH-5 (10 μM) in DMSO or infected with Ad-dnAkt (100 MOI). Ad-GFP (100 MOI) served as a control. Two hours after treatment with SH-5 or 24 h after infection, cells were treated with WISP-1 for an additional 48 h. Protein synthesis and DNA levels were quantified as described above.

Effects of WISP-1 on CFs. The influence of WISP-1 on mouse CF proliferation was analyzed by CyQuant assay (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol (18). Briefly, second-passage CFs were plated into 96-well plates at 3,000 cells/well and allowed to attach overnight. After 24 h, the cells were fed with serum-free medium containing 0.5% BSA and incubated for an additional 24 h. Cells were then continuously stimulated with WISP-1 or PBS vehicle (controls) in serum-free medium for the indicated times. The effect of WISP-1 on collagen expression in CF was determined by Sircol Collagen assay. The Sircol assay (Biorad, Newtowabbey, N. Ireland) is based on the specific binding of the anionic dye, Sirius red, to the basic amino acid residues of collagen (34, 47). Briefly, second-passage CFs were plated into 100-mm culture dishes at 80% confluence, allowed to attach overnight, and refed the following day with serum-free medium containing 0.5% BSA. After 24 h, CFs were stimulated with WISP-1 or PBS vehicle in serum-free medium for 24 to 96 h. Levels of soluble collagens released into culture media by CFs incubated with either WISP-1 or PBS vehicle were determined by spectrophotometry according to the manufacturer’s instructions. Each collagen assay included standard curves using purified soluble collagen supplied by the manufacturer.

Statistical analysis. The number of mice required for each study time point was determined using power analysis. Comparisons between experimental groups were made using the unpaired t-test with Bonferroni’s correction for multiple comparisons for in vivo analysis and two-way ANOVA for in vitro analysis. A P value <0.05 was considered significant. Each experiment was performed three to eight times, and group data were expressed as means ± SE.

RESULTS

MI stimulates adaptive changes in the heart characterized by early elaboration of proinflammatory cytokines and ultimately results in tissue remodeling. Members of the CCN growth factor family (e.g., CTGF) are known mediators of this process. Previous studies have demonstrated WISP-1 transcripts in the heart under basal conditions, though its role, function, and significance are unclear (24). Our results demonstrate for the first time that MI induced by permanent ligation of the LAD results in S-T elevation (Fig. 1A). As shown in Fig. 1, B and C, significantly elevated WISP-1 mRNA levels were detected by 6 h post-MI and were sustained throughout the 7-day study period. Although WISP-1 transcripts were detectable at low basal levels in both naive and sham-operated animals, they showed no evidence of induction. Significant WISP-1 protein upregulation was observed by 24 h, and levels remained elevated thereafter (Fig. 2, A and B). Sham-operated animals showed no time-dependent changes in myocardial signal.
WISP-1 protein levels though this growth factor was detectable at low levels throughout the study period, confirming previous reports (24). Our results show that WISP-1 is expressed at low basal levels and is upregulated in the postinfarcted myocardium.

Biglycan is a small leucine-rich proteoglycan expressed in many tissues in vivo, including skin, kidney, and heart (56), and in vitro by skeletal myocytes, aortic smooth muscle, and endothelial cells (3, 31, 40). We investigated biglycan gene expression in the heart to determine whether this TGF-β-inducible (4a, 54), WISP-1-binding element was regulated post-MI. As shown in Fig. 3, biglycan induction occurred by 6 h in the hearts of infarcted animals and remained elevated for at least 24 h, whereas sham-operated controls showed no upregulation. These data indicate that this WISP-1 binding proteoglycan is induced contemporaneously with WISP-1, demonstrating that the elements for WISP-1 signaling are present in the postinfarcted myocardium.

Since the elaboration of proinflammatory cytokines in the heart following MI is well established (7, 25), and IL-1β is a known inducer of CCN family growth factors including CTGF, the possibility that these mediators may also play a role in WISP-1 upregulation was investigated. As shown in Fig. 4A, significantly elevated myocardial IL-1β and TNF-α transcripts were observed by 3 h post-MI. Treatment of NRVM in culture with either IL-1β (Fig. 4B) or TNF-α (Fig. 4C) alone was sufficient to upregulate WISP-1 protein expression in vitro, demonstrating for the first time that cardiac myocytes express this growth factor. Surprisingly, the data shown in Fig. 4D indicate that TNF-α was more potent than IL-1β at stimulating WISP-1 expression. Treatment with both proinflammatory cytokines together did not result in an induction beyond TNF-α alone, demonstrating that WISP-1 protein expression was maximally sensitive to the TNF-α activation pathway. The specificity of cytokine-induced WISP-1 protein expression was verified since their stimulatory activities could be blocked by coincubation with TNF-α and IL-1β neutralizing antibodies, and cotreatment with control IgG was without effect. These data demonstrate that cytokines are induced post-MI, NRVM express WISP-1, and TNF-α and IL-1β are potent inducers of WISP-1.

WISP-1 has been shown to activate the Akt/PKB survival pathway in some cells (51). Since Akt activation is also involved in hypertrophic signaling (19), we investigated the possibility that WISP-1 was capable of stimulating Akt-dependent myocyte hypertrophy in vitro. As shown in Fig. 5A, we found that treatment of NRVM with WISP-1 resulted in significant cellular hypertrophy as evidenced by increased protein synthesis. Furthermore, the data shown in Fig. 5B demonstrate that WISP-1 stimulates the phosphorylation and activation of
WISP-1 is prohypertrophic and profibrotic growth factor, which is rapidly expressed in the heart post-MI, may have unique functions acutely following myocardial injury. We found that cardiac myocytes not only respond to WISP-1 by undergoing hypertrophy but also express this growth factor rapidly upon stimulation with either TNF-α or IL-1β, two proinflammatory cytokines known to be upregulated acutely in the heart post-MI (7). Thus, in addition to the previously described roles of WISP-1 in tumorigenesis (57), bone development, and fracture repair (26), we show for the first time that in addition to fibroblasts, cardiac myocytes may be a significant source and target for this poorly understood growth factor in the heart.

WISP-1 overexpression in normal skin fibroblasts stimulates proliferation and induces morphological transformation (24). We observed a significant increase in CF proliferation and accompanying increases in collagen deposition following WISP-1 treatment by 48 h in vitro. WISP-1 was shown to bind to small leucine-rich proteoglycans in the extracellular matrix, including biglycan and decorin, leading to the activation of integrin receptor-mediated signaling pathways through complex interactions that are not completely understood (24). Ahmed et al. (1) reported biglycan upregulation in the heart following MI. Biglycan was also shown to be expressed by NRVM in response to iron loading (44), and its upregulation has been demonstrated in CFs from rats with heart failure (1). Consistent with these reports, we found the upregulation of both WISP-1 and biglycan in the myocardium post-MI. Given
that biglycan and WISP-1 are induced in the heart post-MI in vivo and that both NRVM and fibroblasts respond to WISP-1 in vitro, the substrate for WISP-1 upregulation, binding, and activity is present and suggests that WISP-1 signaling may constitute an important adaptive mechanism in the injured heart.

In addition to the proliferative and profibrotic effects on primary CFs, we found that WISP-1 treatment of NRVM stimulated significant myocyte hypertrophy. Although WISP-1 binding has been shown thus far to be restricted to fibroblasts (24) and many of the reported effects of CTGF were described in fibroblast-like cells, we found that WISP-1 potently activated Akt in NRVM. Activation of Akt is known to be important in stimulating cellular hypertrophy and is generally considered to be a prosurvival factor (13, 23). WISP-1 was shown to inhibit p53-mediated apoptosis by blocking cytochrome c release and increasing the expression of B-cell leukemia/lymphoma extra long (Bcl-xL), verifying its prosurvival effects in some cells (51). Interestingly, biglycan, which has been shown to be regulated by TGF-β, may itself be protective since this proteoglycan was reported to enhance the survival of neurons (33, 35). CTGF, which is expressed secondarily to TGF-β and angiotensin II in the heart post-MI, signals through Akt similarly to WISP-1 (20, 55). However, CTGF has been shown to induce apoptosis in several cell types, including human aortic smooth muscle (30) and peritoneal mesothelial cells (53). Thus Akt activation is a shared feature of several CCN family members, including WISP-1 and CTGF though their effects on cell survival may differ (30, 32, 51, 53). Whether these differences are simply cell-type specific or represent bona fide differences in the effects of CCN family growth factors remains unknown.

We found further potentially significant differences with respect to the role played by proinflammatory cytokines and the temporal expression of these growth factors in the heart post-MI. For example, whereas TNF-α was shown to block TGF-β-induced CTGF expression (38) and CTGF-induced fibrosis in several cell types (2), we found TNF-α to be a potent inducer of WISP-1 in myocytes. We and others have shown this cytokine as well as IL-1β to be rapidly upregulated in the heart post-MI (7, 25). Interestingly, we found TNF-α to be a more potent inducer of WISP-1 in myocytes than IL-1β. The possibility that WISP-1 may act early during the acute phase of myocardial injury is suggested not only by its responsiveness to TNF-α and IL-1β but also by the observation that cAMP induction by β-adrenergic agonists or forskolin treatment was shown to block CTGF expression (14). Catecholamines increase rapidly in the heart post-MI, leading to elevated cAMP levels that then decrease gradually over time (15, 39, 41). We observed rapid upregulation of WISP-1 in the heart post-MI where transcripts appeared as early as 6 h and protein levels were significantly elevated by 24 h. This induction occurred at a time when elevated cAMP levels would likely preclude CTGF expression. Interestingly, Xu et al. (57) reported that the cAMP-response element-binding site located in the WISP-1 promoter was important for its induction. Thus the differences between CTGF and WISP-1 induction may offer insights into the unique role played by each factor in the heart post-MI. WISP-1 may act at an earlier stage when proinflammatory cytokines and catecholamines are at maximal levels where it may play a role in myocyte cell survival and growth. At later stages of the healing process, both CTGF and WISP-1 and other factors may contribute in concert to fibrosis and compensatory hypertrophy.

WISP-1 is a target of the Wnt/Frizzled pathway and has been shown to be regulated by β-catenin (57). However, Wnt-independent mechanisms have been demonstrated in cardiomyocytes, which lead to inactivation of GSK-3 and result in β-catenin stabilization, nuclear translocation, and β-catenin-regulated cardiomyocyte growth (28). It is plausible that an additional consequence of β-catenin stabilization would be WISP-1 induction. Interestingly, it was recently shown that TNF-α treatment stabilizes β-catenin in adipocytes (5). It is possible that a similar mechanism may be responsible for our observation that TNF-α potently induces WISP-1 in cardiomyocytes. Furthermore, we found that WISP-1 itself results in Akt activation and phosphorylation of GSK-3 substrate at Ser21 (GSK-3α) and/or Ser9 (GSK-3β), which, at least in the case of GSK-3β, has been shown to result in its inactivation (50). Thus WISP-1 may participate in its own expression by inhibiting GSK-3β, stabilizing β-catenin, and resulting in β-catenin responsive gene expression. Additional experiments will be needed to definitively establish a role for GSK-3β- in TNF-α-induced WISP-1 induction.

Our results offer new insights into the potentially important role of WISP-1 in the heart post-MI. We found that both WISP-1 and biglycan are rapidly upregulated in the injured heart and, with respect to the former, levels remain elevated for at least several days. Furthermore, WISP-1 has significant effects on myocyte growth where it rapidly and potently activates Akt, resulting in myocyte hypertrophy. In addition, we show that WISP-1 is similar to other CCN family members in stimulating CF proliferation and collagen release. Significantly, these data indicate that WISP-1 is unique in that it is potently induced by TNF-α in myocytes. Taken together, our results suggest a possible mechanism by which MI rapidly upregulates WISP-1 and biglycan in the heart, resulting in increased WISP-1 signaling leading to myocardial hypertrophy, fibroblast proliferation, and fibrosis. Although WISP-1 is perhaps the least understood member of the CCN growth factor family, these data should stimulate further investigations into the role of this potentially important factor in the heart.

GRANTS

This work was supported in part by the Research Service of the Department of Veterans Affairs (to B. Chandrasekar); National Heart, Lung, and Blood Institute Grants HL-68020 (to B. Chandrasekar) and HL-67971 (R. Mestril); and American Heart Association Texas Affiliate Beginning Grant-in-Aid 0565018Y (to J. T. Colston).

REFERENCES


