Cardiotrophin-1 Stimulation of Cardiac Fibroblast Growth
Roles for Glycoprotein 130/Leukemia Inhibitory Factor Receptor and the Endothelin Type A Receptor


Abstract—Cardiotrophin-1 (CT-1), a member of the interleukin-6 superfamily, and endothelin-1 (ET-1) are potent hypertrophic factors in cardiomyocytes. Although CT-1 and ET-1 gene expression in the heart is upregulated in experimental heart failure, their role in the activation of the cardiac fibroblast is unknown. This study was designed to identify the presence and action of CT-1 and its receptor complex, glycoprotein130 (gp130) and leukemia inhibitory factor (LIF) receptor, on cardiac fibroblast growth in cultured adult canine cardiac fibroblasts. In addition, we investigated the interaction between CT-1/gp130/LIF receptor and ET-1/endothelin type A (ETA) receptor axis. Immunohistochemistry was performed using the indirect immunoperoxidase method, while we assessed the cell cycle of cardiac fibroblasts by flow cytometry, DNA synthesis by [3 H]thymidine incorporation, and collagen synthesis by [3 H]proline incorporation, respectively. CT-1 and gp130/LIF receptor were widely present in the cytoplasm of the cardiac fibroblasts. Exogenous CT-1 markedly stimulated [3 H]thymidine and [3 H]proline incorporations (P<0.01), with accumulation of cells in the S phase. Blockade of gp130 or LIF receptor inhibited basal growth as well as CT-1– or ET-1–stimulated cardiac fibroblast growth. The specific ETA receptor antagonist, BQ123, significantly inhibited CT-1–stimulated DNA synthesis. This study demonstrates that CT-1 and its receptors are present in cardiac fibroblasts. In addition, growth of these cells stimulated by endogenous and exogenous CT-1 requires gp130/LIF receptor as well as ETA receptor activation. We conclude that gp130/LIF receptor and ETA receptor activation are essential for cardiac fibroblast growth by CT-1 and that there is synergism with ET-1/ETA receptor axis. (Circ Res. 2002;90:128-134.)

Key Words: cardiac fibroblast ■ cardiotrophin-1 ■ glycoprotein130 ■ leukemia inhibitory factor receptor ■ endothelin-type A receptor

Many lines of evidence have revealed that several humoral factors, including angiotensin II (Ang II) and endothelin-1 (ET-1), contribute to the progression of cardiac hypertrophy and congestive heart failure in the adaptive process.1,2 These pathological states are accompanied by cardiomyocyte growth, fibroblast proliferation, and collagen accumulation. Importantly, excessive fibrosis causes a reduction in diastolic compliance, resulting in impaired myocardial function and heart failure. Cardiotrophin-1 (CT-1) has recently been identified as a member of the interleukin (IL)-6 super family, which has growth-promoting properties in cardiomyocytes.3 CT-1 binds to glycoprotein130 (gp130) and the leukemia inhibitory factor (LIF) receptor, a component of the gp130 receptor.4 Two signaling pathways, signal transducer and activator of transcription and mitogen-activated protein kinase (MAPK), have been identified.5 CT-1 gene expression is present in various organs, including the heart,6 and it has been reported that CT-1 gene expression is increased in ventricular myocardium after myocardial infarction (MI)7 and in hypertensive rats.6,8 We recently reported that cardiac CT-1 gene expression is activated in both canine atrial and ventricular myocardium in experimental congestive heart failure.9 Based on these data, CT-1 may have an important role in the development of cardiac hypertrophy and heart failure. However, the presence of CT-1 and its receptor complex on the cardiac fibroblast is unknown, as is its action on cardiac fibroblast growth.

ET-1 and Ang II are growth promoters in both cardiomyocytes and cardiac fibroblasts.10–12 ET-1 is activated following MI and in hypertensive heart disease.13,14 It has been reported that ET-1, derived from cardiac fibroblasts, mediates cardiac hypertrophy via endothelin type A (ETA) receptor by a paracrine mechanism.15 Both CT-1 and ET-1 mediate via MAPK on growth5,16 but the interaction between CT-1/gp130/LIF receptor axis and the endothelin system remains unknown.

The current study was designed to identify the presence and action of CT-1 and its receptor in cultured adult canine...
cardiac fibroblasts. In addition, we investigated the interaction of the CT-1/gp130/LIF receptor complex and the ET-1/ET_A axis on cardiac fibroblast growth. Our goal was to provide new insights into the biology of CT-1 in cardiac fibroblasts including potential cross-talk.

Materials and Methods

Cell Culture

Normal adult canine ventricular cardiac fibroblasts were prepared according to the method described by Weber et al17 with some modification.18 In brief, the left ventricular free wall was excised from an adult mongrel dog (Antech, Inc, Barnhart, Mo) anesthetized with sodium pentobarbital. The tissue was rinsed with cold phosphate-buffered saline (PBS) and minced with surgical scissors into small pieces, and then digested with 0.12% trypsin and 0.03% collagenase for 8 periods of 10 minutes in each. The collected cells were preincubated in Medium 199 (M199) (BioWhittaker) with 10% fetal bovine serum (FBS) at 37°C for 2 hours to allow nonmyocyte cells (mostly cardiac fibroblasts) to attach to the bottom of culture dishes. After decanting of the medium, the cells were cultured with fresh M199 supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B in 5% CO_2/95% humidified air at 37°C. Antibodies for gp130, LIF receptor, or ET_A receptor antagonist, or a tyrosine kinase inhibitor, genistein, or a MAPK inhibitor, PD98059, were added to the culture medium 30 minutes before adding CT-1 or ET-1. We used the cardiac fibroblasts within three passages in this experiment. The cultured cells exhibited positive staining for vimentin and negative staining for von Willebrand factor, α-smooth muscle actin, and α-sarcemeric actin, indicating that there was no relevant contamination of our cardiac fibroblast culture with endothelial cells or smooth muscle cells or cardiac myocytes (data not shown). This study conformed to the guidelines of the American Physiological Society and was approved by the Mayo Clinic Animal Care and Use Committee.

Immunohistochemistry

Immunohistochemical analysis for CT-1, gp130, LIF receptor, and ET-1 was performed as previously described.19 In brief, cultured cardiac fibroblasts were fixed with 4% paraformaldehyde for 20 minutes and washed 3 times with PBS. To block the endogenous peroxidase, cells were incubated with 0.6% hydrogen peroxide in methanol for 20 minutes at room temperature. After washing the cells with PBS, they were incubated with 5% normal goat serum for 10 minutes at room temperature to reduce the nonspecific background. Then, they were incubated with rabbit polyclonal antibodies for CT-1 (Research Diagnostics Inc, Flanders, NJ), gp130, and LIF receptor, and for ET-1 (Phoenix, Belmont, Calif) at a dilution of 1:100 at room temperature for 24 hours. Cells were incubated with a second antibody-horseradish peroxidase conjugate for 30 minutes at a dilution of 1:100. Visualization was achieved by incubating the cells with freshly prepared reagent containing 3-amino-9-ethylcarbazole (Sigma) dissolved in N,N-dimethylformamide, hydrogen peroxide, and sodium acetate. Cells were also counterstained with hematoxylin and examined under the microscope (Olympus). The specificity of the antibodies for gp130 and LIF receptors was confirmed by substitution of nonimmune rabbit serum, absorption testing, and Western blotting.

Flow Cytometry

After 48 hours of incubation with 1% FBS containing M199 medium in 6-well culture plates, we further incubated cells with fresh 1% FBS containing M199 medium in the absence or presence of 10^{-4} mol/L of CT-1 for 24 hours. Cells were harvested by trypsinization and rinsed in PBS, and the DNA content was assessed by flow cytometric measurement of propidium iodide fluorescence. Fraction of cells in G0/G1, S, and G2/M phase was estimated using Becton Dickinson FACScan flow cytometer with ModFit software. A minimum of 10 000 cells was analyzed to determine the percentage of cells in each phase.

Proliferation Assay

The rate of DNA synthesis was assayed by measuring [3H]thymidine in the acid-insoluble fraction of the cells as previously described.18 Collagen synthesis was assessed by [3H]proline incorporation into cells. After reaching 60% to 70% confluence in 24-well culture plates, M199 medium containing 1% FBS was changed and incubated for 48 hours. Fibroblasts were then exposed to CT-1 with or without antibodies for CT-1, gp130, LIF receptor, or BQ123, or genistein, PD98059, in the fresh medium. Cells were pulsed with 1.0 μCi/mL of [3H]proline for 40 hours and then washed three times with cold PBS and incubated with 10% trichloroacetic acid at 4°C for 20 minutes. After cell residues were rinsed with 95% ethanol, the dried materials were solubilized in 0.5 N NaOH for 2 hours. Radioactivity of the solubilized materials was determined using a liquid scintillation counter (LS-6000SC, Beckman Inc). The results were divided by protein content and expressed as the percent of control. The protein content was measured by Lowry's method.20

Western Blotting

For detection of translocation, the cardiac fibroblasts were harvested according to previous reported methods21 with minor modifications. Briefly, cells were homogenized by sonication in homogenization buffer (10 μL of each proteinase inhibitor: 5 mg/mL leupeptin, 10 mg/mL aprotinin, 1 μmol/L pepstatin, 100 μmol/L phenylmethylsulfonyl fluoride in 1 mL of solution containing 250 mmol/L Sucrose, 10 mmol/L ethylene glycol-bis[β-aminoethyl ether]-N,N',N''-tetraacetic acid, 2 mmol/L ethylenediaminetetraacetic acid, and 20 mmol/L Tris-HCl, pH 7.4, and centrifuged (14 000 rpm) for 15 minutes. The supernatant was collected as a cytosol fraction. The pellet was sonicated with homogenized buffer described above with 1% Triton X-100 and centrifuged at 14 000 rpm for 15 minutes. The supernatant was collected as a particulate fraction. For immunoblotting, 30 μg of denatured protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 7.5% polyacrylamide gel (Bio-Rad), and separated proteins were electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad). Nonspecific binding was blocked by incubation with 5% nonfat dry milk overnight and Bohringer blocking buffer (0.5% Bohringer blocking agent, 10 mmol/L Trizma-base, 150 mmol/L NaCl, pH 7.4) at room temperature for 2 hours. The PVDF membranes were then incubated with the anti-gp130 polyclonal antibody (1:2000) or anti-LIF receptor polyclonal antibody (1:2500) for 1 hour at room temperature. After washing in Tween-20 buffer, the membranes were incubated with goat anti-rabbit horseradish peroxidase (1:10 000) for 1 hour at room temperature. The immunoreactive bands were visualized by ECL Plus detection (Amersham).

Reagents

Recombinant human CT-1 was purchased from Research Diagnostics, Inc (Flanders, NJ); endothelin-1 and BQ23 from Phoenix (Belmont, Calif); Angiotensin II and monoclonal anti-α-sarcomeric actin from Sigma; monoclonal antibodies for vimentin, α-smooth muscle, and von Willebrand factor from DAKO (Carpinteria, Calif); Genistein and PD98059 from Calbiochem; [Methyl-3 H]thymidine and [3H]proline from Amersham Pharmaic Biotech; and antibodies for rabbit polyclonal gp130, LIF receptor, and goat anti-rabbit horseradish peroxidase-linked secondary antibody from Santa Cruz Biotechnology.

Statistical Analysis

Unpaired Student’s t test was used for comparison between 2 groups. Multiple comparisons were assessed with 1-way ANOVA followed by Scheffe’s test. All data were expressed as mean±SEM of the samples examined and a value of P≤0.05 was considered significant.
Results

Immunohistochemical Staining for CT-1, gp130, LIF Receptor, and ET-1 in Canine Cardiac Ventricular Fibroblasts

Positive staining was observed for CT-1 and its receptors, gp130 and LIF receptor, in canine cardiac fibroblasts. Representative immunohistochemical stainings are illustrated in Figure 1. Positive immunoreactivity was widely distributed in the cytoplasm of cells. ET-1 immunoreactivity was distributed in a similar pattern to CT-1 and its receptors. The cells treated with preabsorbed excessive peptides demonstrated little or no immunoreactive stainings for these antibodies (data not shown).

Effect of CT-1 on Cell Cycle, De Novo DNA, and Collagen Synthesis

Figure 2 illustrates the effects of CT-1 on the cell cycle (A) by flow cytometric measurement, and [3H]thymidine (B) and [3H]proline (C) incorporation. CT-1 (10^-8 mol/L) increased the percentage of cells in S phase 4-fold (P<0.01). In addition, 10^-8 mol/L of CT-1 significantly (P<0.01) stimulated [3H]thymidine and [3H]proline incorporation into the cells by 186% and 21%, respectively.

Dose Responses of CT-1, Ang II, and ET-1 on De Novo DNA and Collagen Synthesis in Cardiac Fibroblasts

Figure 3A illustrates the dose-response of CT-1 on DNA synthesis in cardiac fibroblasts. CT-1 markedly (P<0.01) stimulated [3H]thymidine uptake into cells in a dose-dependent manner between 10^-11 and 10^-8 mol/L, with maximal effects at 10^-8 mol/L. Although 10^-7 mol/L of Ang II or ET-1 induced DNA synthesis in these cells (Figures 3B and 3C), the effect of CT-1 was much greater. CT-1 also significantly (P<0.01) stimulated [3H]proline incorporation into the cells between 10^-11 and 10^-8 mol/L (Figure 3D).
Effect of Antibodies for gp130, LIF Receptor, or CT-1 on the CT-1 Action

Figures 4A to 4D illustrate the effects of antibody blockade of gp130 or LIF receptor as well as CT-1 on basal and CT-1–induced cardiac fibroblast growth. Pretreatment with antibodies for gp130 or LIF receptor significantly (P<0.01) inhibited basal and CT-1 (10⁻⁶ mol/L)–induced [³H]thymidine incorporations. As shown in Figure 4C, these antibodies showed a similar inhibitory effect on [³H]proline incorporation. In addition, antibody for CT-1 also significantly inhibited basal (P<0.05) as well as CT-1–stimulated (P<0.01) [³H]thymidine incorporation (Figure 4D).

Effect of Inhibitors for Tyrosine and MAPK on CT-1–Stimulated Cell Growth

Figures 5A to 5D illustrate the effect of genistein, a tyrosine kinase inhibitor, and PD98059, a MAP kinase inhibitor, on basal and CT-1 (10⁻⁸ mol/L)–stimulated DNA and collagen synthesis. Pretreatment with these inhibitors (10⁻³ mol/L) significantly (P<0.01) inhibited basal and CT-1–stimulated DNA synthesis, respectively. In addition, these inhibitors also had a significant inhibitory effect of collagen synthesis by CT-1.

Effect of ETₐ Receptor Antagonism on CT-1–Stimulated DNA Synthesis

Figure 6A illustrates the action of BQ123, a specific ETₐ receptor antagonist, on ET-1–stimulated DNA synthesis. BQ123 significantly (P<0.01) inhibited not only basal (ie, by endogenous ET-1) but also DNA synthesis by exogenous ET-1. In addition, BQ123 significantly (P<0.01) attenuated CT-1–stimulated DNA synthesis between 10⁻⁵ mol/L and 10⁻⁶ mol/L (Figure 6B).

Effect of Anti-gp130 or LIF Receptor Antibody on the ET-1 Action

Figures 7A and 7B illustrate the effect of antibodies for gp130 or LIF receptor on basal and ET-1–stimulated [³H]thymidine incorporation. These antibodies significantly (P<0.01) inhibited the basal as well as ET-1–stimulated DNA synthesis, respectively.

Localization of gp130 and LIF Receptor in Cytosolic and Particulate Fraction

Figures 8A and 8B illustrate the distribution of the band for gp130 and LIF receptor in the cardiac fibroblasts. gp130 was detectable as a band of 13 kDa and was present in the cytosol as well as particulate fraction, whereas neither CT-1 or ET-1 changed the intensity of the band. LIF receptor was also recognized as a band for 19 kDa and present predominantly in the particulate fraction. The band of cytosolic as well as particulate fraction was enhanced by treatment with 10⁻⁷ mol/L ET-1.

Figure 4. Inhibitory effects of antibodies for gp130, LIF receptor, or CT-1 on basal and CT-1–stimulated DNA and collagen synthesis in the cardiac fibroblasts. Values are shown as mean±SEM (A and B, n=4; C, n=6; D, n=12 in IgG and anti–CT-1 Ab; n=6 in IgG+CT-1 and Anti–CT-1 Ab+CT-1). Radioactivity in the control cells was 1697±60, 2237±12, 1193±31, and 4354±150 DPM/µg protein, respectively. *P<0.05, **P<0.01, compared with controls incubated with nonspecific immune rabbit IgG; ##P<0.01, compared with cells incubated with 10⁻⁸ mol/L CT-1.
Discussion

Cardiac fibroblasts, which represent 25% of the volume and 70% of the total cell number of the heart,22,23 serve a vital role in preserving myocardial structure. In addition, they importantly participate in the progression of ventricular remodeling.24 The objective of the current study was to advance our understanding of cardiac fibroblast biology and specifically determine the presence of the hypertrophic factor CT-1 and its receptor complex (gp130 and LIF) in cultured canine cardiac fibroblasts. We also sought to reveal the growth promoting properties of CT-1 on fibroblasts and the crosstalk with the ET-1/ET_A receptor system. The present findings establish the presence of CT-1, gp130, and LIF receptor immunoreactivity in cardiac fibroblasts by immunohistochemistry. We also report the modulating prospective of CT-1 and ET-1 on fibroblast growth and the importance of the gp130/LIF receptor as well as ET_A receptor on the action of CT-1.

Aoyama et al7 have reported that increased CT-1 and gp130 immunoreactivities are detectable in both the cytoplasm and the perinuclear region of cardiomyocytes and cardiac fibroblast-like cells after MI, and translocation of gp130 occurs from cytoplasm to the cell surface, suggesting that CT-1 and its receptor may participate in ventricular remodeling. In addition, CT-1 gene expression has been reported to be more intense in neonatal rat cardiac fibroblasts, compared with that of cardiomyocytes.25 Although it was suggested that release of CT-1 from cardiac fibroblasts might be important for the growth of cardiomyocytes, the action of CT-1 on cardiac fibroblasts has remained unknown.

Our findings confirm and extend these previous reports and demonstrate that exogenous CT-1 stimulates DNA and collagen synthesis with the accumulation of cells in the S phase. Although Ang II and ET-1 have been reported to be important growth promoters in cardiac fibroblasts, the action of these peptides on adult cardiac fibroblasts in culture were less than those of previous studies using neonatal rat cardiac fibroblasts.11,12,18,26 Importantly, we observed that CT-1 exhibited a much stronger activity compared with these 2 other vasoactive peptides.

Recently, Talwar et al27 have reported that plasma CT-1 concentration in normal humans is 10^{-11} to 10^{-10} mol/L, and increases to 10^{-10} to 10^{-9} mol/L in patients with left ventricular systolic dysfunction. Furthermore, CT-1 gene expression has been reported to increase in the infarcted ventricular myocardium2 and in hypertensive rats, both of which are accompanied by both cardiomyocyte hypertrophy and hyperplasia of the interstitium.6,8 Based on these data, CT-1 may act on fibroblast growth under both pathological and physiological conditions.

CT-1 has been reported to act via the heterodimerization of gp130 and the LIF receptor–signaling subunit.4 Indeed, we report that not only blocking CT-1 by anti–CT-1 antibody but also blocking heterodimerization by pretreatment with either the antibody for gp130 or LIF receptor significantly inhibited the actions of exogenous CT-1. In addition, these antibodies also inhibited basal levels of DNA and collagen synthesis in cardiac fibroblasts. Although antibodies used in this study

Figure 6. The effect of BQ123, a specific ET_A receptor antagonist, on basal and ET-1 or CT-1-stimulated DNA synthesis in the cardiac fibroblasts. Values are shown as mean±SEM (A and B, n=4). Radioactivities in the control cells were 1831±81 and 2203±85 DPM/μg protein, respectively. *P<0.05, **P<0.01, compared with controls; ***P<0.05, ****P<0.01, compared with the cells incubated with 10^{-9} mol/L CT-1.
were not monoclonal for selective detection of gp130 or LIF receptor by Western blotting (data not shown), these data suggest that endogenous CT-1, which may be secreted from cardiac fibroblasts, may act at least in part as a growth factor in an autocrine/paracrine mechanism via gp130/LIF receptor complex. Consistent with this concept is the observation that mice lacking gp130 display a lethal and deficient phenotype showing defects affecting ventricular myocardial development.28

Recently, marked cardiac distention induced by aortic constriction was reported in a murine gp130 knockout model, in which the mutation was confined to the ventricular myocardium.29 In addition, it has been reported that CT-1 is antiapoptotic and cytoprotective,30,31 and that mice lacking the LIF receptor exhibit placental, skeletal, neural, and metabolic defects that are lethal in the perinatal phase.32 Considering these data, our results are consistent with the emerging concept that CT-1 and gp130/LIF receptor complex on the cardiac fibroblast may be vital for interstitial maintenance of myocardial structure. By contrast, excessive CT-1 in pathological conditions such as MI or hypertensive heart disease may stimulate fibroblasts to produce extracellular matrix proteins and thus cause excessive cardiac fibrosis. Further studies investigating this speculation are warranted.

Finally, we have elucidated an interaction between CT-1/gp130/LIF receptor complex and ET-1/ETA receptor axis on DNA synthesis, which has not been reported to date. ET-1, derived from cardiac fibroblasts, has been known to mediate a hypertrophic response via the ETA receptor in cardiomyocytes.15 In addition, CT-1 and ET-1 share a common signal transduction system, which includes MAPK activation.5,16 Saito et al53 have reported that ANP- and β-MHC–luciferase activation by ET-1 was inhibited when cardiomyocytes were transfected with a dominant negative mutant of gp130. Our hypothesis that CT-1 activation of DNA synthesis would couple to ET-1/ETA receptor in the cardiac fibroblast was confirmed by the findings that the specific ETA receptor antagonist, BQ123, inhibited not only ET-1– but also CT-1– stimulated DNA synthesis.

To date, the interaction between cytokines and vasoactive peptides in the heart remains to be fully characterized. The relationship of the local angiotensin system and IL-6 family including CT-1 has been reported to be involved in the progression of cardiac hypertrophy.34,35 In addition, it is reported that tumor necrosis factor-α augments Ang II type 1 receptors in neonatal rat cardiac fibroblasts.36 In this study, kinases for tyrosine and MAP are involved for growth of cardiac fibroblasts by CT-1. Secondly, pretreatment with antibodies for gp130/LIF receptor abolished the action of ET-1. Thirdly, our study importantly indicates that ET-1 stimulates the translocation of LIF receptor from cytosol to cell surface. Our data suggest that an interaction between CT-1/gp130/LIF receptor and ET-1/ETA receptor may occur in cardiac fibroblasts, and cross-talk between these 2 systems is of importance in cardiac fibroblast activation.

All IL-6 family members, such as IL-6, CT-1, LIF, oncostatin M, and ciliary neurotrophic factor, require gp130 as a signal-transducing subunit and all share similar biological actions.5,37 Our in vitro studies suggest that their binding to gp130 and LIF receptor may affect endogenous proliferative stimulatory actions. In addition, we used 1% FBS in the experiments. We therefore cannot exclude the possibility of an effect of serum on CT-1 action. It should be noted that it remains unknown whether the in vitro data in the current study can be extended to the in vivo situation. Our study in a canine model of early dilated cardiomyopathy produced by progressively increasing pacing rates (180 to 200 bpm for approximately 14 to 20 days)38 demonstrates that ventricular CT-1 and gp130 are decreased and LIF receptor is enhanced, as compared with normal, by immunohistochemical staining (data not shown). Considering the important role of cardiac fibroblasts, which maintain the structure of the heart, this in vivo finding suggests that impaired CT-1 protein and gp130 axis in this model could contribute to progressive ventricular remodeling. Further studies will be required to clarify the role of CT-1 and its potential cross-talk with ET-1 in the intact heart. Nonetheless, the current studies support the concept that CT-1 and gp130/LIF receptor complex contribute to the regulation of cardiac structure via a mechanism that involves the ET-1/ETA receptor axis.

In conclusion, we report that CT-1 and its receptor complex are present in adult canine cardiac fibroblasts. The action of CT-1 and its receptor complex on cardiac fibroblast growth also involves an important cross-talk with the endothelial system. These findings, therefore, provide important new insights into the biology of the cardiac fibroblast and important interactions between cytokines and vasoactive peptides.

Acknowledgments

This research was supported by grant HL-36634 from the National Institute of Health, by the Miami Heart Research Institute, by the Mayo Foundation, and by the Bruce and Ruth Rappaport Program in Vascular Biology. T. Tsuruda was supported by the fellowship of Japan Heart Foundation. We gratefully thank Denise M. Heublein and Sharon M. Sandberg for their technical assistance and Linda Combs for her assistance in preparing the manuscript.

References


Cardiotrophin-1 Stimulation of Cardiac Fibroblast Growth: Roles for Glycoprotein 130/Leukemia Inhibitory Factor Receptor and the Endothelin Type A Receptor


Circ Res. 2002;90:128-134; originally published online December 13, 2001;
doi: 10.1161/hh0202.103613

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/90/2/128

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/