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Tissue Inhibitor of Metalloproteinase-4 Instigates Apoptosis in Transformed Cardiac Fibroblasts

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Abstract Tumor cells become malignant, in part, because of their activation of matrix metalloproteinases (MMPs) and inactivation of tissue inhibitor of metalloproteinases (TIMPs). Myocardial tumors are rarely malignant. This raises the possibility that the MMPs and TIMPs are differentially regulated in the heart compared to other tissues. Therefore, we hypothesized that a tissue specific tumor suppressor exists in the heart. To test this hypothesis we prepared cardiac tissue extracts from normal (n = 4), ischemic cardiomypathic (ICM) [n = 5], and dilated cardiomyopathic (DCM) [n = 8] human heart end-stage explants. The level of cardiospecific TIMP-4 was determined by SDS-PAGE and Western-blot analysis. The results suggested reduced levels of TIMP-4 in ICM and DCM as compared to normal heart. TIMP-4 was purified by reverse phase HPLC and gelatin-sepharose affinity chromatography. Collagenase inhibitory activity of chromatographic peaks was determined using fluorescein-conjugated collagen as substrate and fluorescence spectroscopy. The activity of TIMP-4 (27 kDa) was characterized by reverse zymography. The role of TIMP-4 in cardiac fibroblast cell migration was examined using Boyden chamber analysis. The results suggested that TIMP-4 inhibited cardiac fibroblast cells migration and collagen gel invasion. To test whether TIMP-4 induces apoptosis, we cultured cardiac normal and polyomavirus transformed fibroblast cells in the presence and absence of TIMP-4. The number of cells were measured and DNA laddering was determined. The results suggested that TIMP-4 controlled normal cardiac fibroblast transformation and induced apoptosis in transformed cells. Cardiospecific TIMP-4 plays a significant role in regulating the normal cell phenotype. The reduced levels of TIMP-4 elicit cellular transformation and may lead to adverse extracellular matrix degradation (remodeling), cardiac hypertrophy and failure. This study suggests a possible protective role of TIMP-4 in other organs which are susceptible to malignancy. J. Cell. Biochem. 80:512–521, 2001. © 2001 Wiley-Liss, Inc.

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Harris and Watkins [1965] infused normal cells with transformed cells and observed that the resulting phenotype that came out in the fused cells was of normal cells. Therefore, they concluded that tumor-suppressor genes must exist in the normal tissue and cells. Activation of matrix metalloproteinases (MMPs) play a significant role in the development of malignant tumor. The cancer cell degrades the basement membrane by activation of MMPs and spreads into the blood stream and metastasizes to the other organs [Liotta, 1992]. The levels of tissue inhibitor of metalloproteinases (TIMPs) are repressed in malignant tumors [Zucker et al, 1999].

In normal heart TIMP-4 is constitutively expressed [Greene et al., 1996] and repressed in heart failure [Mujumdar and Tyagi, 1999; Tyagi, et al., 1993]. Unlike other organs cardiac tumors rarely metastasize. Therefore, tissue-specific control mechanisms must exist against tumor malignancy in the heart and TIMP-4 is thought to control the normal cardiac cell phenotype, otherwise the reduced levels of TIMP-4 may lead to the development of a malignant phenotype and cardiac failure.

Extracellular matrix (ECM) sounding the cardiomyocytes protects the cardiac muscle

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function. A single myocyte contracts and relaxes but it synchronizes only when connected with other myocytes by ECM. When adult cardiomyocytes are prepared by digesting the tissue with collagenase, these myocytes do not live past 3–4 h after disruption of surrounding ECM milieu. It may suggest that ECM degradation triggers the apoptotic cascades in cardiac muscle [Meredith et al., 1993; Juliano and Haskill, 1993], presumably due to decreased levels of TIMP-4. Levels of TIMP-4 increases during compensatory ECM remodeling and hypertrophy and decreases in decompensatory adverse ECM remodeling, apoptosis, and heart failure [Mujumdar and Tyagi, 1999]. This suggested a protective role of TIMP-4 in the compensatory phase during cardiac remodeling. Heart tissue is resistant to invasion by cancer cells [Le Marer and Bruyneel, 1996]. It is possible that transformed cell proliferation and growth inhibitory activity in cardiac extract is due, in part, to the presence of TIMP-4 in the normal heart [Tyagi et al., 1999]. We hypothesize that the reason cardiac tumors are rarely malignant is in part due to the presence of high levels of constitutively expressed TIMP-4 which controls the cardiac cell transformation, therefore, it prevents cardiac malignancy. The reduced levels of TIMP-4 may lead to a malignant phenotype and organ failure.

MATERIALS AND METHODS

Source of Tissue

Human heart tissue was obtained at the time of cardiac transplant in the operating room at the University of Mississippi Medical Center. We obtained tissue from failing hearts from 5 patients with documented coronary artery disease and myocardial infarction (ischemic cardiomyopathy, ICM); and 8 patients from idiopathic dilated cardiomyopathy (DCM). The coronary arteries in DCM hearts were normal. Four normal samples were obtained from the donor hearts. The average age of the infarcts were 4 ± 1 years. The severity of heart failure was identified by the NYHA heart failure classification. The NYHA score were three for ICM and four for DCM patients. Tissue specimens from infarcted, DCM and normal hearts were collected and processed within 1–2 h [Tyagi et al., 1996a].

Preparation of Cardiac Tissue Homogenate

The cardiac tissue extracts from respective tissues were prepared as described by Tyagi et al. [1993]. The total protein in the samples was estimated using a Bio-Rad dye binding assay. The proteins in cardiac extract were separated into < 50 and > 50 kDa fractions by dialyzing with 50,000 Dalton cut-off membrane.

SDS-PAGE and Western-blot Analysis for TIMP-4

SDS-PAGE was prepared according to Laemmli [1970]. To determine the difference in the mobility and to keep the subunits intact, the gels for protein separation were carried under non-reducing condition and stained with Coomassie blue. Gels for Western-blot analysis were performed under reducing condition as described by Tyagi et al. [1996b]. After electrophoresis the gel was transferred onto nitrocellulose paper. The band specific for TIMP-4 was blotted using anti-TIMP-4 antibody (Chemical Corp.).

HPLC and Affinity-Chromatography Separation of TIMP-4

Cardiac tissue homogenates were dialyzed against 20 mM KH_2PO_4/K_2HPO_4 (pH 7.0), and separated by reverse-phase chromatography. TIMP-4 was isolated by typically injecting 0.5 ml (2 mg protein) of the dialyzed pool through a C18, 5 μM, 100 Å (Waters Corp. Milford, MA) and eluted over 30 min, 3.9 mm × 150 mm; flow 1 ml/min, mobile phase 35% v/v 20 mM KH_2PO_4/K_2HPO_4 at pH 7.0/ 65% methanol at 24°C. The components of major chromatographic peaks were resolved by SDS-PAGE and analyzed for collagenase inhibitory capacity to identify fractions containing TIMP-4 protein. The peak containing collagenase inhibitory activity was further purified by gelatin-sepharose chromatography and Bio-Rad Prep cell methods. Bound fractions were eluted with 0.5 M NaCl [Tyagi and Cleutjens, 1995]. The TIMP-4 was identified by reverse zymography and immuno-blot analyses. The concentration of purified TIMP-4 was determined based on the absorbance at 280 nm of 6.5, which is equivalent to 10 mg/ml of protein.

Collagenase Inhibitory Activity of TIMP-4

Twenty micrograms of cardiac collagenase was activated for 5 min at 37°C with 5.5 μM

trypsin. Trypsin was subsequently inhibited by the addition of 7 mM PMSF. Then 20 μg fluorescein-conjugated collagen (Elastin Product Co. St Louis) was added to the activated collagenase and incubated at 37°C in 50 mM Tris-Cl (pH 7.6), 5 mM $CaCl_2$, and 0.03% NaN_3. Prior to the addition of collagen, TIMP-4 containing fractions were added to the activated collagenase. The fluorescein-collagen fragments were analyzed on 7.5% SDS-PAGE and fluorography under UV illumination. Also, TIMP-4 fractions were analyzed using fluorescence substrate (suc-GPGLP-AMC) as described by Tyagi et al. [1995b] and by reverse zymography [Tyagi et al., 1993]. The bands on reverse zymographic gels were compared with the bands on Western-blot analysis.

Cardiac Normal and Transformed Fibroblast Cells

Cardiac fibroblasts were isolated and characterized [Tyagi et al., 1995a]. These cells were positive for vimentin but they were negative for α-actin, suggesting non-muscle origin of these cells. Cardiac fibroblasts were transformed by polyoma virus middle T-antigen as described by Kaplan et al. [1986] and Tyagi et al. [1996c]. These cells grow rapidly and have been shown to express an increased amount of active MMPs as compared to normal cardiac fibroblasts. The cells were grown to confluence in 10% fetal bovine serum, 4.5 mg/ml glucose, gentamycin and fungizone (10 μg/ml), and 2 mM glutamine. The cells were incubated at 37°C in an water jacketed incubator and then humidified with 90% O_2/10% CO_2. The cells were washed two times with serum-free Dulbecco's modified Eagle's minimal essential medium (MEM) prior to the experimental treatment.

To measure the cell migration and collagen gel invasion, a Boyden chamber apparatus was employed as described by Tummalapalli and Tyagi [1999]. In upper well thin collagen gel was placed on the top of 8 μm filter paper. The native collagen gels were prepared using type I collagen from rat tail tendons (Southern Biochemical Corp.). Concentrated MEM and bicarbonate buffer solutions were mixed 1:1 with the collagen suspension (3.1 mg/ml) on ice to yield the final isotonic collagen gel solution (pH 7.4) according to the manufacturer's instructions. In some collagen gels 10 μg/ml TIMP-4 was incorporated. Serum deprived cells were placed on top of the gel. In the lower chamber 10 μg/ml basic FGF was added to MEM for chemoattractive activity. In control experiment lower chamber contain equal concentration of BSA. After different time intervals, the gels were stained with coomassie blue for lytic activity of migratory cells.

For cell growth measurements, equal concentrations of normal and transformed cells were plated on six-well plates. To each well different dose of TIMP-4 was added. The cells were counted by hemocytometer at 0, 48 and 72 h after the treatment of TIMP-4. To determine specificity of TIMP-4 and to reverse the inhibition of transformed cell growth, the transformed cells were pre-incubated with anti-TIMP-4 antibody at 1:200 dilution. The cells were detached and counted. The total genomic DNA was isolated. The DNA was analyzed on 1.2% agarose gel electrophoresis using Triethanolamine buffer. The DNA fragments were analyzed under UV transluminator.

Inhibition of transformed fibroblast proliferation by TIMP-4 was carried out using in vitro cell culture. The number of cells were counted by measuring acid phosphatase activity as the marker of cell proliferation before and after the treatment with TIMP-4 in 96-well microplate reader. Transformed and normal cardiac fibroblasts were proliferated by serum and/or bFGF in culture medium as growth promoter.

Proliferation of normal cardiac fibroblasts and transformed cells were carried out as described by Tyagi et al. [1995a, 1996c]. Fibroblast cells 2×10^3 were plated onto gelatin-coated 96-well tissue culture dishes (Nunc) on Day 1. On Day 2, cells were fed with Dulbecco's modified Eagle's medium (Gibco) with 5% calf serum (Hyclone) (DMEM/5), and increasing concentrations of TIMP-4. Wells containing phosphate-buffered saline (PBS) (Gibco) alone and PBS + bFGF with DMEM were included as controls. On Day 5, media was removed and cells were washed with PBS. Acid phosphatase activity in these cells was measured as the marker of proliferation and cell population [Connolly et al., 1986]. This assay exhibited a linearity between acid phosphatase activity and fibroblast cell number up to 10,000 cells per well. We verified this linearity in the presence of TIMP-4 and other known inhibitors of fibroblast cell proliferation such as copper-bound low molecular weight heparin [Raju et al., 1984], vitreous, and platelet factor-4.

Percent inhibition was determined by comparing wells exposed to stimulus with those exposed to stimulus and inhibitor (TIMP-4).

RESULTS

TIMP-4 is Suppressed in End-Stage Heart Diseases

To determine the levels of TIMP-4 in ICM (n = 5) and DCM (n = 8) hearts, we performed non-reducing SDS-PAGE and Western-blot analyses. In parallel control normal (n = 4) heart tissue was examined. An apparent band (~27 kDa) present in the normal heart tissue was nearly absent in ICM and DCM hearts (arrow, Fig. 1A). The Western-blot analysis demonstrated the presence of TIMP-4 in the normal heart and no significant levels of TIMP-4 in end-stage heart failure (Fig. 1B), suggesting that the levels of TIMP-4 were suppressed in heart disease.

Regulation of Growth of Transformed Fibroblast Cells by Myocardial Inhibitor

Extracts from normal myocardium inhibited the growth of polyoma transformed fibroblast cells more than that of normal fibroblasts. Extract from cartilage also inhibited the growth but the extract from skin did not. Normal cardiac extract was fractionated by dialyzing with a cut-off membrane of 50 kDa. The fraction <50 kDa demonstrated growth inhibitory activity against transformed cells. These result suggest that repression of growth of transformed cell by myocardial inhibitor is, in part, due to an inhibitor of molecular weight <50 kDa.

Isolation and Purification of TIMP-4

To purify TIMP-4 from normal cardiac tissue, we performed reducing SDS-PAGE and ran HPLC analysis. The reducing SDS-

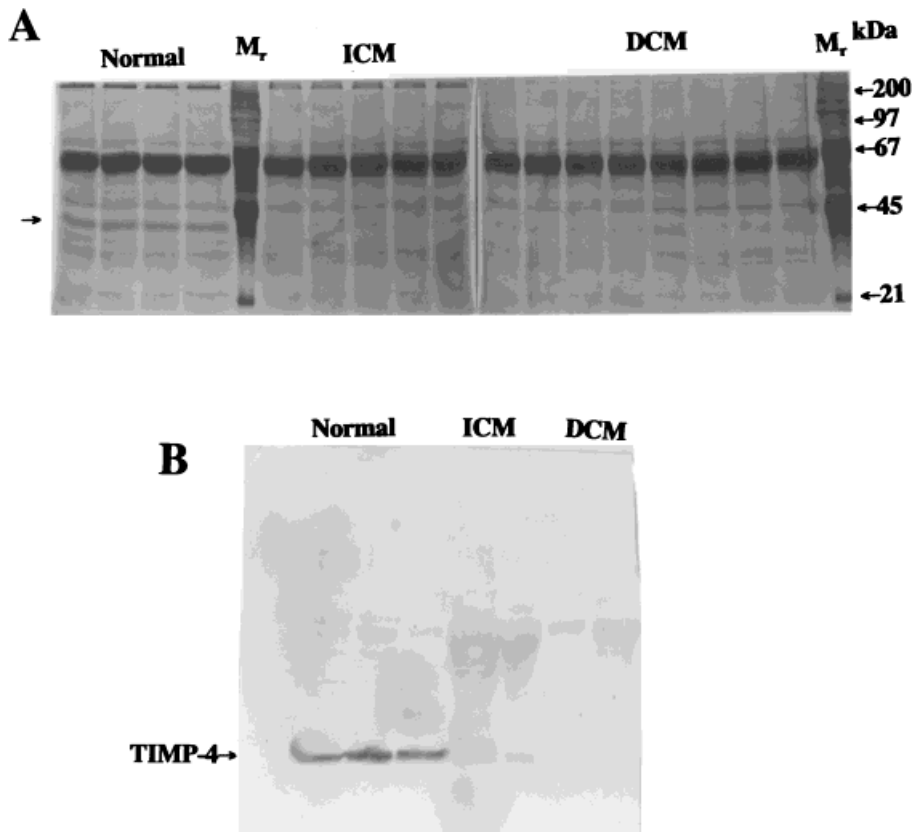

Fig. 1. Non-reducing differential SDS-PAGE analysis of cardiac tissue homogeneates from normal (n = 4), ischemic dilated cardiomyopathy (ICM) (n = 5), and dilated cardiomyopathy (DCM) (n = 8). Molecular weight markers (M_r) are shown in the middle and on the right of the gel. Below is the representative Western-blot of TIMP-4 (**B**); normal (n = 3); ICM (n = 2) and DCM (n = 2).

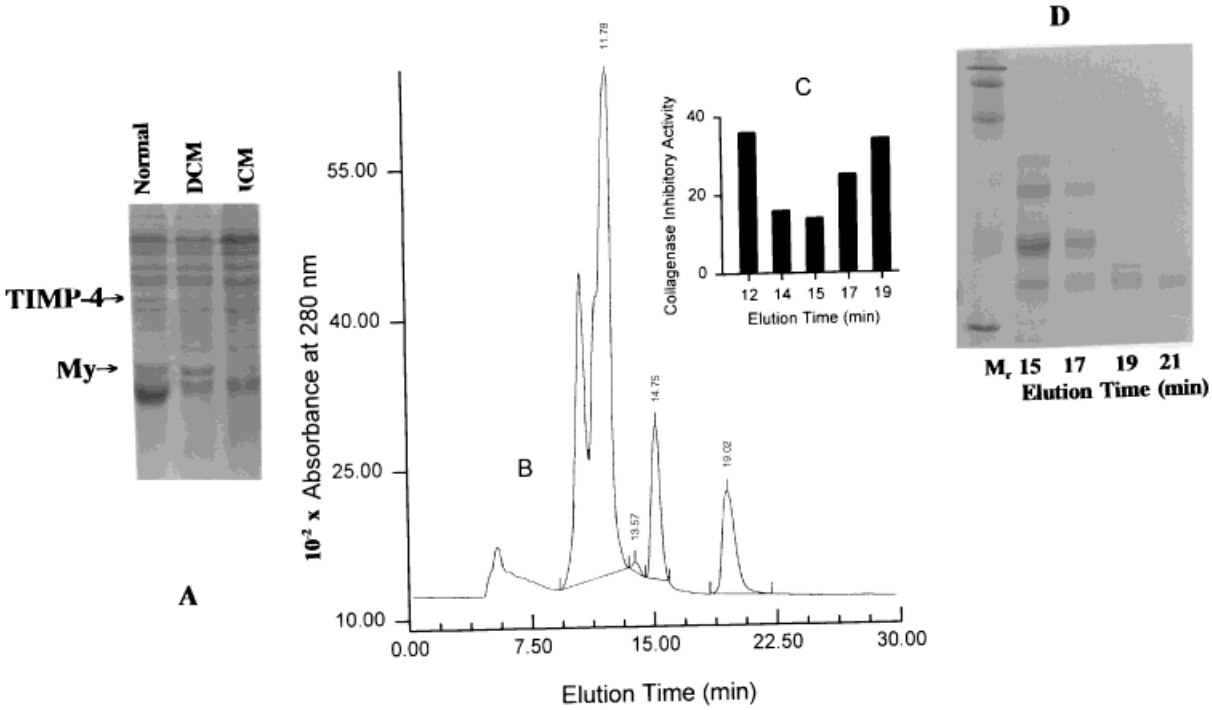

Fig. 2. Purification of cardiac TIMP-4 from normal human heart tissue extract: (**A**) reducing differential SDS-PAGE analysis of normal, DCM and ICM (arrow indicate TIMP-4). A protein of ~27 kDa (arrow) is present in normal heart and absent in infarcted and DCM hearts; (**B**) HPLC profile of normal heart extract. The extract was run through a reverse phase C-18 column. The peaks were analyzed for collagenase inhibitory activity; (**C**) collagenase inhibitory capacity of HPLC isolated peaks is shown in (C). Bars represent the degree of collagenase inhibition from HPLC separated fractions. Standard purified cardiac collagenase was used with selective fluorogenic substrate (Suc-GPGLP-AMC). Collagenase activity was monitored using florescence at 438 nm when excited at 380 nm due to substrate hydrolysis. The graph indicates collagenase inhibitory capacity of the peaks isolated from HPLC at different elution time. Peak at 12 and 19 min demonstrated maximum collagenase inhibitory activity. Peak at 12 min is probably due to MMP/TIMP complex and peak at 19 min is due to TIMP. (**D**) SDS-PAGE analysis of HPLC peaks.

PAGE demonstrated an extra band at ~27 kDa in normal heart. This band was near absent in DCM and ICM hearts. Note that myoglobin band (My) is also decreased in ICM heart (Fig. 2A). The HPLC analysis separated in many peaks. The peaks for TIMP-4 activity were analyzed for collagenase inhibitory activity (Fig. 2B). The results demonstrated two collagenase inhibitory fractions. The peak at 12 min is probably due to MMP/TIMP-4 complex and peak at 19 min is most probably free TIMP-4 (Fig. 2C). The analysis of these fractions on SDS-PAGE suggested that the peak at 19 min contains an extra band observed in normal heart (Fig. 2D).

The HPLC fractions between 17 and 21 min were further purified on gelatin-sepharose chromatography. The fraction eluted with 0.5 M NaCl were collected and analyzed by Western-blots for TIMP-4 (Fig. 3). The results suggested that the TIMP-4 was a single subunit of a molecular weight of ~27 kDa.

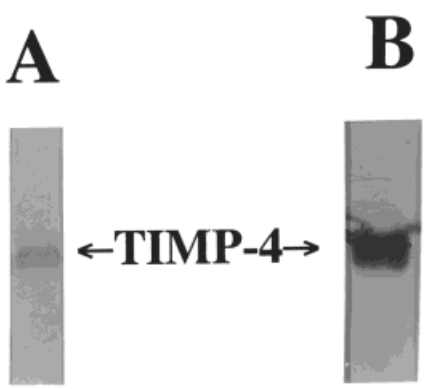

Fig. 3. Peak containing TIMP-4 activity was further purified on gelatin-affinity column. Peak isolated by 0.5 M NaCl was analyzed by SDS-PAGE (**A**). (**B**) TIMP-4 was analyzed by Western-blot of peak shown in A.

The level of TIMP-4 in the failing heart was reduced significantly as compared to the normal heart. This suggested that the reduced levels of TIMP-4 in the heart may induce a malignant phenotype in cardiac fibroblast cells.

The isolation and purification of TIMP-4 produced enough quantity of TIMP-4 to determine the role of TIMP-4 in inhibition of transformed fibroblast cell growth.

Role of TIMP-4 in the Inhibition of Invasion of Collagen Gel Matrix

It is known that migratory cells produce active MMPs and this MMP activity is required for invading the matrix barrier. To determine whether inhibition of MMPs in the migratory cardiac fibroblast cell will inhibit its invasion, we performed cell invasion assays. The results suggested that FGF chemoattractant deriven fibroblast cell invasion was inhibited by TIMP-4 (Fig. 4). This activity of TIMP-4 was abolished when TIMP-4 was mixed with anti-TIMP-4 antibody, suggesting specific role of TIMP-4 in inhibition of fibroblast cell invasion. Similar results were obtained for transformed fibroblast cells.

Inhibition of Growth of Transformed Fibroblasts by TIMP-4

The growth of normal and transformed cardiac fibroblast cells were measured in the presence and absence of TIMP-4. The results suggested that TIMP-4 has no effect on growth of normal cardiac fibroblasts. However, the growth of transformed fibroblasts was attenuated by TIMP-4 (Fig. 5). The DNA analysis in these cells demonstrated significant DNA damage in transformed cells as compared to normal fibroblast cells (Fig. 6). The number of viable cells was decreased in transformed cells as compared to normal cells after culturing these cells with TIMP-4. This activity of TIMP-4 was reversed by anti-TIMP-4 antibody (Fig. 7).

To examine the specificity of this inhibition by TIMP-4, we tested other substances for their ability to inhibit fibroblasts proliferation. Other enzyme inhibitors such as trypsin ovoinhibitor, pancreatic trypsin inhibitor, α2-macroglobulin (a collagenase inhibitor), and dermatan sulfate (a glycosaminoglycan found in the heart) were examined. These inhibitors do not have significant effect on growth-factor-stimulated fibroblast proliferation, even at high concentration. This suggested that TIMP-4 is indeed a specific inhibitor of tumor cell proliferation. The inhibitory effect of

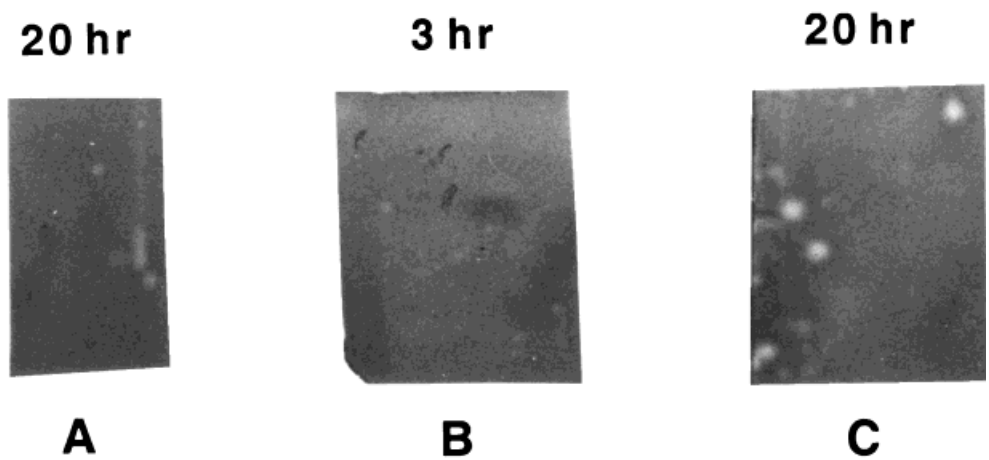

Fig. 4. Light micrograph of cultured fibroblast cells invading onto type I collagen gel containing bFGF (a chemoattractant): Inhibition of bFGF-driven fibroblast cell migration and invasion through collagen gel by TIMP-4 (**A**). Fibroblast cells migrated normally (B, 3 h; and C, 20 h) through collagen gel without TIMP-4. Lytic activity demonstrates migration of fibroblast cells through basement membrane coating in Boyden chamber. The coating was created uniform with collagen barrier with thickness of ~10 micron. Approximately 1000 cells were laid on to the top of the collagen gel and incubated for 3 and 20 h under the gradient of basic-FGF. Cells migrated in aggregated form. After incubation collagen gel was stained with coomassie blue. The time-dependent degradation of collagen barrier is shown at 3 h (**B**) and more at 20 h (**C**) due to the lytic activity of fibroblasts expressing MMP during passing through the matrix barrier. This activity of MMP was inhibited by TIMP-4 in collagen gel (A). Collagen gel was prepared with 8 vol of Vitrogen-100 collagen (Collagen Corp, Palo Alto, CA) mixed with 1 vol of 0.1 M NaOH and 1 vol of 1.5 M PBS at 4°C with and without 10 μg/ml TIMP-4. Collagen mixture was allowed to gel for 2–4 h at 37°C. Thereafter, 1 ml culture medium containing 1×10^3 cells was added on the top of the gel and incubated at 37°C. Cell migrated into the gel were identified by staining gel with coomassie blue for lytic activity. Cell migration through the gel was time-dependent and was more over 20 than 3 h. This chemoattractant-driven migratory and invasive activity of fibroblast was inhibited by TIMP-4.

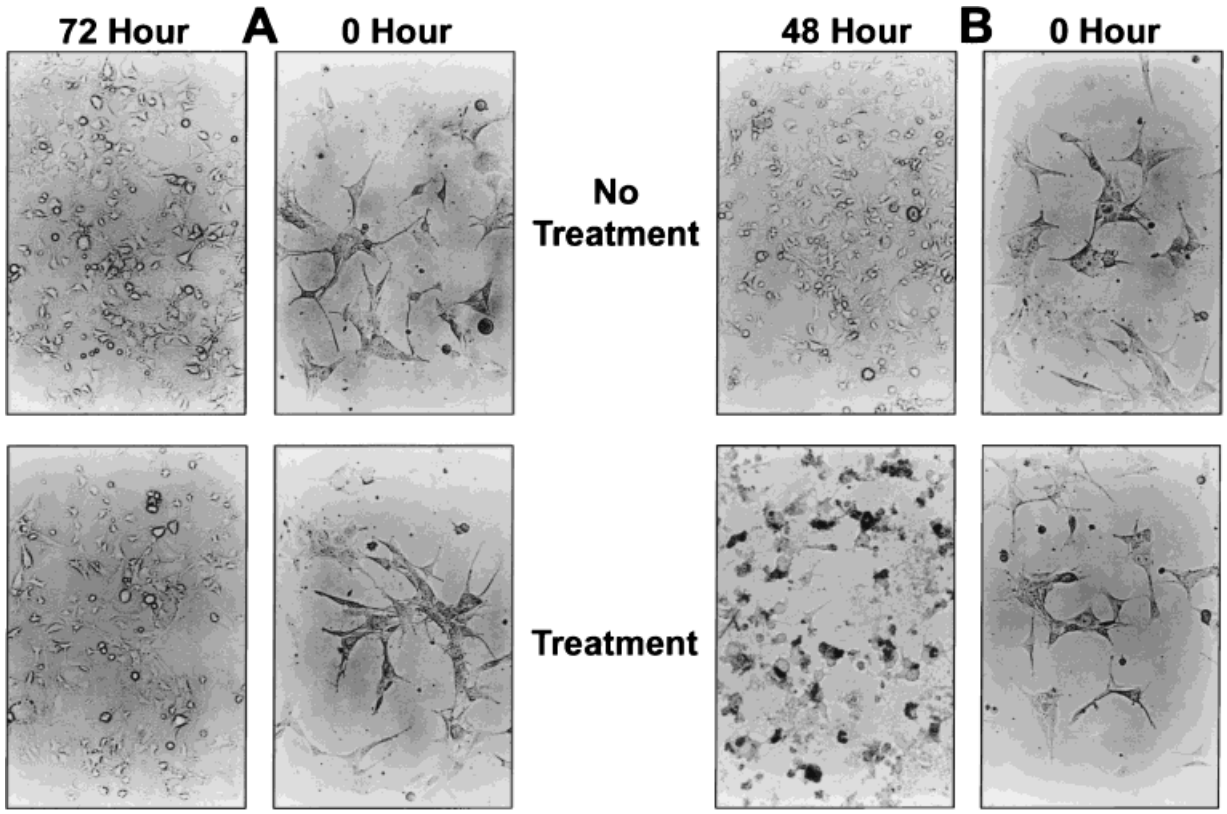

Fig. 5. Inhibition of growth of transformed cardiac fibroblasts at 48 h treatment with purified TIMP-4: **A, upper left panel** demonstrates growth of normal fibroblasts without TIMP-4; **A, lower left panel**, with TIMP-4 (10 μg/ml). **B, upper left panel** demonstrates growth of transformed cardiac fibroblasts without TIMP-4; **B, lower left panel**, with TIMP-4 (10 μg/ml). The 5% serum containing DMEM was used as culture medium. TIMP-4 did not alter significantly the proliferation of normal heart fibroblasts up to 72 h of culture, suggesting that TIMP-4 has no effect on normal fibroblast cells. On the other hand, the number of transformed fibroblasts was reduced significantly after the treatment with TIMP-4 within 48 h. This suggested that TIMP-4 suppresses tumor cell growth and proliferation.

TIMP-4 did not have a significant effect on non-fibroblastic cells, such as smooth muscle cells, or Balb/c 3T3 cells (American Type Culture Collection). This further suggested that TIMP-4 is a specific inhibitor for transformed cardiac fibroblast cell proliferation.

DISCUSSION

TIMP-4 is a cardiospecific inhibitor of MMPs, however, its role in cardiac function is not well understood. We demonstrated that cardiac TIMP-4 contains anti-proliferating, anti-migratory and anti-invasive property against transformed fibroblast cells. TIMP-4 induced apoptosis in transformed fibroblasts and not in normal fibroblasts.

A single cell multiplies, changes phenotype, and forms different tissue types, during normal development. However, in certain tissues some cells become tumorigenic and malignant. Malignancy is the ability of a tumor cell to grow progressively and kill their host. Tumor growth and abnormal neovascularization are the life threatening conditions. The presence of angiogenic factors in tumors undergoing neovascularization are found in many normal tissues where neovascularization is not occurring. Accumulated evidence indicates that in most tumors, the switch to angiogenic phenotype depends upon the outcome of a balance between angiogenic simulators and angiogenic inhibitors, both of which may be produced by normal and transformed cells. The evidence suggests physiological expression of angiogenic stimulating factors is tightly regulated by angiogenic inhibitors. Understanding of the tissue specific angiogenic stimulator and inhibitor are the key elements in understanding the mechanism of tumor malignancy.

Under normal conditions of physiological angiogenesis, new blood vessel formation, cell

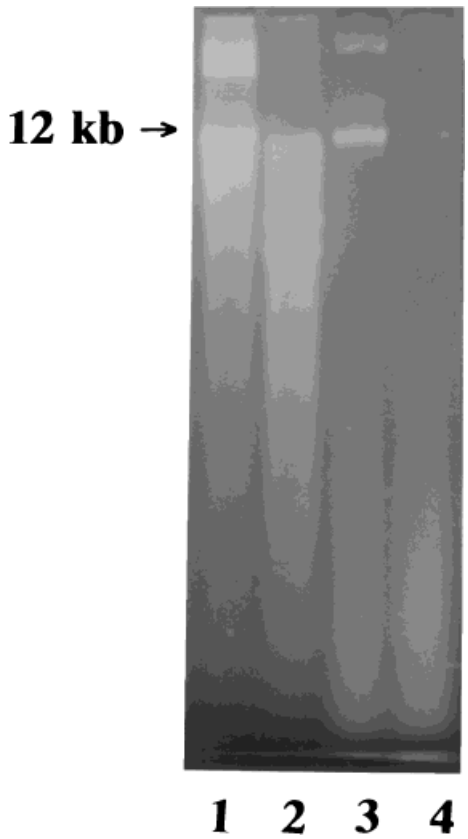

Fig. 6. DNA analysis of cell treated with TIMP-4: Lane 1, normal cells treated with TIMP-4 for 48 h; lanes 2, 3 and 4 are transformed cells treated for 30 min, 12 and 24 h with TIMP-4.

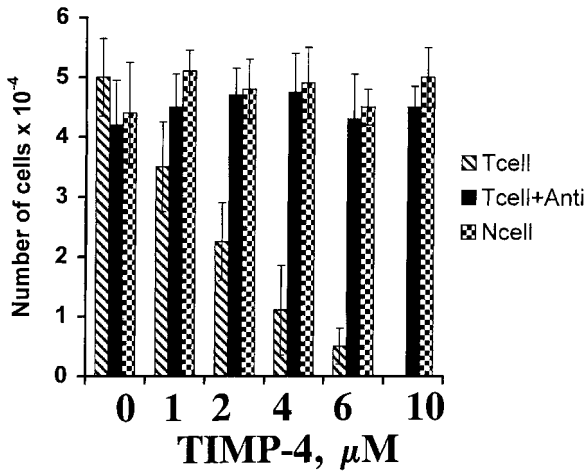

Fig. 7. Cell growth of normal (N_{cell}) and transformed (T_{cell}) fibroblasts with or without the treatment of TIMP-4: Normal cells grew with treatment or without treatment. The growth of transformed cell was reduced significantly ($P < 0.01$) in the presence of 6–10 μM TIMP-4 as compared to cell without treatment. All cells at confluence were divided to equal number (200); N_{cell}, T_{cell}, and T_{cell} treated with anti-TIMP-4 (1:200 dilution) [T_{cell} + anti] prior to incubation with different concentration of TIMP-4 for 72 h in 0.5% FCS. After 72 h cells were detached by trypsin and counted using hemocytometer. Each experiment was carried out in triplicates and mean ± SD is reported.

growth and proliferation are associated with wound healing, corpus luteum formation, and embryonic development [Nagase and Woessner, 1999; Woessner, 1991]. MMPs play an important role in normal physiological remodeling [Tyagi, 1997]. However, MMPs are also responsible for tumor metastasis (i.e. degradation of basement membrane, leading to escape of tumor cell into blood stream and to invade other tissues) [Liotta, 1992]. MMPs are stringently controlled by tissue specific TIMPs [Tyagi et al., 1995b]. Eighty four percent of heart tumors are benign and rarely malignant [Grande et al., 1993]. Majority of the myocardial tumors are myxomas, fibroelastomas and fibrosarcoma [Kapoor, 1986]. The histological analysis of heart tumors suggest the presence of endothelial cells, macrophages, mature or immature smooth muscle cells, fibroblasts, and elastic fibers covered by an abundant acid mucopolysaccharide matrix [Kapoor, 1986]. Extracts from tissues, such as cartilage is one of the few avascular tissues which can inhibit angiogenesis and growth of tumor cell. Extracts from cartilage inhibit proliferation of cultured fibroblast cells [Eisenstein et al., 1975]. Chick heart tissue inhibits invading activity of human breast carcinomas cells [Le Marer and Bruyneel, 1996]. Although extracts from several different tissue sources have been shown to exhibit anti-angiogenic activity [Folkman et al., 1988], no single tissue-derived macromolecule in the heart capable of inhibiting tumor growth and angiogenesis has been identified. We demonstrated that TIMP-4 is, in part, responsible for inhibition of growth of transformed cardiac fibroblasts and malignancy of cardiac tumors.

A differential role of TIMPs in cellular function has been suggested. For example, TIMP-1 has been shown to demonstrate antimitogenic activity [Hayakawa et al., 1992; Moses, 1991; Moses and Langer, 1991]. TIMP-2 has been shown to be a growth stimulatory protein for transformed fibroblasts [Nemeth and Goolsby, 1993]. We previously have shown that TIMP-1 has proliferative activity to endothelial cells [Tyagi et al., 1996d]. Baker et al. [1998] have demonstrated that TIMP-3

induces apoptosis in vascular smooth muscle cells and regression of the neointimal growth. Here, we demonstrated that cardiospecific TIMP-4 induces apoptosis in transformed cardiac fibroblast cells and does not effect normal cardiac fibroblast cells.

Perspective

This study elucidated the role of TIMP-4 in controlling cell growth and inhibition of tumor malignancy in the heart and have implications for other organs which are susceptible to malignancy. To identify the anti-metastatic mechanism and agent responsible for tumor repression, the development of method to identify early detection of metastatic tumors, is inevitable. In this regard, the measurements of plasma TIMP-4 in cancer or pre-cancer patients should be beneficial. TIMP-4 is highly expressed in heart than any other tissue [Greene et al., 1996]. Levels of TIMP-4 are increased in compensatory hypertrophy and reduced in decompensatory heart failure [Mujumdar and Tyagi, 1999]. To determine the physiological role of TIMP-4 in normal heart, we purified cardiac TIMP-4. It is of great interest to determine the role of TIMP-4 in cardiac hypertrophy. The ex vivo contractile studies using cardiac ring preparation and purified cardiac TIMP-4 are in progress.

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