RESEARCH ARTICLE

Curcumin Involve the Myofibrosis Process of Rabbit Valve Interstitial Cells based on Expression Alpha- Smooth Muscle Actin: Experimental Posttest-only Control Group Design

IGN Iswan R. Ranuh,* J. N. E. Putranto, Achmad Lefi

Department of Cardiology and Vascular Medicine, Specialist Medical Education Program-1 Faculty of Medicine, University of Airlangga, Rumah Sakit Umum Daerah Dr. Soetomo, Surabaya, Indonesia

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ABSTRACT

Introduction: Following an acute rheumatic fever infection brought on by an inflammatory reaction to streptococcal bacteria, rheumatic heart valve disease results in valve degeneration. Elements of the immune system play an important role, in facilitating myofibroblast transdifferentiation and clearing damaged tissue of apoptotic cells. Myelofibrosis arising in valve components is the main cause of valve dysfunction. Valve myelofibrosis showed a significantly increased collagen, proteoglycan, and elastin content in myofibrotic valves compared to healthy valves. Objective: To establish curcumin's capacity to prevent rabbit valve interstitial cells (VIC) from differentiating into myofibroblasts after being stimulated by TGF-1 by comparing the results to controls.

Method: This study uses a posttest-only control group design for an *in-vitro* laboratory experiment. Heart VIC of a New Zealand rabbit were isolated (*Oryctolagus cuniculus*), and induced fibrosis by administration of TGF-β1 5 ng/mL. VIC pretreated with TGF-β1 were treated with low-dose curcumin (20 nanoM/L) and high-dose curcumin (50 nanoM/L). The immunocytochemical method based on SMA expression observed the inhibition of myofibroblastic differentiation. Statistical significance was analyzed by Kruskal-Wallis statistical test with a p-value <0.05 as the limit of significance.

Result: Administration of curcumin with low doses (20 nanoM/L) and high doses (50 nanoM/L), significantly decreased TGF-β1-induced myofibroblastic differentiation of VIC, which was characterized by decreased SMA expression in all low-dose curcumin treatment groups (20 nanoM/L) (1.40 ± 6.30) and high-dose curcumin (50 nanoM/L) (1.40 ± 8.30).

Conclusion: Curcumin potentially prevent the development of SMA-expressing VIC into myofibroblasts.

Keywords: Curcumin, Myofibroblasts, Smooth muscle actin, Valve interstitial cells.

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INTRODUCTION

Valvular heart disease (VHD) has a disease burden that is projected to increase in the next few decades and is one of the leading causes of morbidity and mortality in cardiovascular disease worldwide. The prevalence of valvular heart disease alone apparently affects about 41 million people worldwide, and this figure is steadily increasing in developing countries. Reflecting the distribution of the disease, rheumatic heart disease remains the most common manifestation of VHD worldwide.[1] The heart valve composition consists of valve endothelial cells (VEC) and valve interstitial cells (VIC) and functions to maintain valve homeostasis and the integrity of the leaflet structure. VIC, which is the most abundant cell type in heart valves, plays a key role in the process of heart valve diseases, one of which is the differentiation of valvular myofibrosis.[2] Inflammatory mediator cytokines play an active role in the differentiation process of myofibrosis. One of the many members of a broad superfamily of inflammatory mediator cytokines is transforming growth factor (TGF), which plays a significant role in activities like proliferation, wound healing, and the synthesis of extracellular matrix (ECM) molecules that accumulate and play a part in the differentiation of fibroblasts.[3] Myofibroblasts themselves have a role in mediating maladaptive responses to injury or injury which will result in excessive accumulation of ECM proteins. Elements of the innate immune system also play an important

**Author for Correspondence: iswanranuh@yahoo.co.id*

role, facilitating myofibroblast trans differentiation and clearing damaged tissue of apoptotic cells and ECM remnants. At the cellular level, myofibrosis arising in valve components is a major cause of valve dysfunction. Myofibrosis of this valve has been demonstrated by Lis Y *et al.* who showed a significantly increased collagen, proteoglycan, and elastin content in myofibrotic valves compared to healthy valves.[4] There is an interesting fact from several previous studies that in rheumatic heart disease conditions, It has been demonstrated that TGF-1 plays a significant function as a cytokine in the induction of myofibroblastic differentiation.[5-8]

Exposure to TGF-β1 can induce SMAD and MAPK signaling pathways with downstream effects in the form of promiofibrosis protein synthesis.[9,10] and cause myofibroblastic differentiation, which functionally can stimulate ECM synthesis twice as potent as precursor cells resulting in valvular myofibrosis.[5,11] Therefore TGF-β1 was chosen as a cytokine in this study to create an *in-vitro* pro-fibrotic inflammation model that might mimic the condition of the valves affected by rheumatic heart disease. In chronic conditions, patients with rheumatic heart disease can adapt to the current symptoms. The development of worsening symptoms is often the main driving indication for either definitive or prophylactic therapeutic intervention. World Health Organization (WHO) recommends long-term administration of antibiotics as secondary prophylactic therapy for rheumatic heart disease. The duration of antibiotic administration is determined by carditis complications that occur in the acute phase of rheumatic fever and the age of the patient with the longest duration of antibiotic administration being lifelong.[12] This long-term antibiotic prophylaxis method is still used as one of the therapeutic efforts in cases of rheumatic heart disease. However, it is unknown whether these efforts can prevent the myofibrosis process in the valves, so trials of alternative prophylactic therapies to prevent myofibrosis in rheumatic heart disease can be a solution.[13] There is new hope for avoiding the myofibrotic transition in rheumatic heart disease since antifibrotic medications can be utilized to stop the differentiation of VIC into myofibroblasts in the early stages of the inflammatory phase (Table 1). The pleiotropic effect of curcumin exhibits antifibrotic potential through inhibition of some TGF-β1 signaling in human ventricular fibroblasts by their receptors on valve interstitial cell surfaces.[14,15] Curcumin appears to be able to suppress the three main activation pathways of TGF-β1 in the Smad-dependent and Smad-independent pathways through inhibition of Smad2/3/4 and ERK phosphorylation, and inhibit the initiation of collagen I and III proteins. In addition, curcumin can stimulate the upregulation of bone morphogenic protein -7 (BMP-7) which this mediator can inhibit TGF-β1, eventually leading to antifibrotic activity.[16] In addition, it has been said that curcumin supplementation reduces TGF-β1 expression through increasing levels of VE-cadherin, DDAH1, and Nrf2 where these mediators can inhibit myofibroblast differentiation and extracellular matrix synthesis.[17] Studies exploring how hepatocytes, renal glomerular cells, and TGF-1 prevent the growth of myofibroblasts,[18] There have been studies using myocytes,[19] pulmonary alveolar cells, and ventricular fibroblasts,[20,21] but none have looked at the antimyofibrotic effectiveness of curcumin on VIC. Based on these factors, this study sought to determine how well curcumin inhibited myofibrogenesis in the early stages of the inflammatory phase in the VIC, to prevent the acute stage of the disease's valve myofibrosis process, protecting individuals from its long-term effects.

Table 1: Mean Quantity of Expression of SMA in Myofibroblasts

Group	*(Minimum-Maximum)*
TGF-β1 +	23474 (18452 - 25671)
TGF-β1 -	1075 (118 - 7696)

METHOD

This research is experimental laboratory research (*in-vitro*) with a post-test control group-only design method. Some of the interventions that will be given are (1) low-dose curcumin (20 uM/L); (2) high dose curcumin (50 M/L). The valvular interstitial cell (VIC), which was stimulated with TGF-1, served as the study's experimental unit. The experimental unit was created using VIC cultures harvested from New Zealand rabbits' valves (*Oryctolagus cuniculus*), then grouped into several groups, namely the group given a dose of curcumin low (20 M/L) and high dose curcumin (50 M/L). The research is planned to be carried out in the stem cell laboratory, Institute of Tropical Diseases, Airlangga University, Surabaya which will be carried out for approximately 3 months. This study used *O. cuniculus* rabbits from New Zealand, 12–13 weeks old, weighing 2.5–3 kg. Data collection on multiple resistances: the first stage was (1) reagent prescription, (2) dissection preparation (3) VIC followed by the second stage: cell culture, the third stage of cell maintenance (maintenance), the fourth stage of valve interstitial cell characteristics, the fifth stage of the experimental protocol, the sixth stage of VIC, differentiation assessment into myofibroblasts. The following move is five times each experiment was run. The primary data obtained will be edited, coded, and entered into a computer using SPSS software version 26.0 for MacOs (SPSS Inc, Chicago, IL). Descriptive analysis is displayed in the form of a median and the Wilcoxon test is performed with = 0.05. Kruskal-Wallis comparative analysis combined with post hoc Mann-Whitney. The difference was considered significant if $p < 0.05$.

RESULT

Average expression quantity of SMA in VIC a median of 23474 after being treated to TGF-1 as a myofibroblastic differentiation inducer (18452 - 25671)

Median expression and quantity analysis of SMA markers in the based on Table 2 treatment groups with expression. The lowest SMA was in the curcumin group with a dose of 50 nanoM/L with a mean of 1.40 ± 8.30. The expression of SMA in the curcumin group at a dose of 20 nanoM/L was 1.40 ± 8.30. Comparative analysis of effects of low-dose curcumin and

Table Treatment Group 2: Quantity of Expression of SMA in Treatment Group

Median	*(Minimum-Maximum)*
Curcumin 20 nanoM/L	3.25 (1.40-6.30)
Curcumin 50 nanoM/L	2.40 (1.40-8.30)
Control	20.60 (15.50 – 35.00)

Table 3: Comparative Analysis of the Effects of Curcumin based on dose on Control

Group Statistic		*Kolmogorov-Smirnov*			
		df	*Sig.*	*Normality of*	
Treatment	20	.122	18	.200	Abnormal
	50	.165	18	0.008	Normal
	Control	.200	18	.239	Normal
Test Kruskal-Wallis		df 2		Asymp. Sig. p .155	

Table 4: Test post-hoc mann-whitney

Group	*Mean Expression*	*Test Mann-Whitney Significance (p-value)*
Curcumin 20 Control	9.50 27.50	Significant (p<0.001)
Curcumin 50 Control	8.70 27.50	Significant (p<0.001)
Curcumin 20 Curcumin 50	19.72 17.28	Not Significant (p<0.486)

high-dose curcumin on control in inhibiting myofibroblastic differentiation based on SMA expression (Table 3).

The differences between treatment groups were analyzed significantly in the post hoc Mann-Whitney test and the Kruskal-Wallis test were employed to assess the data further, and it was discovered that there was a significant difference between the low-dose curcumin (20 nanoM/L), group and the control group ($p < 0.001$) and high-dose curcumin (50 nanoM/L with control ($p < 0.001$), but no difference was found between the low-dose curcumin (20 nanoM/L) group and the high-dose (50 nanoM/L) curcumin group ($p < 0.486$) (Table 4).

From the statistical test results, it can be concluded that the administration of low and high doses of curcumin significantly reduced In the low-dose curcumin intervention group (20 nanoM/L). Based on SMA expression, rabbit VIC were equally potently differentiated into myofibroblasts. additionally high-dose turmeric (50 nanoM/L) (Figure 1).

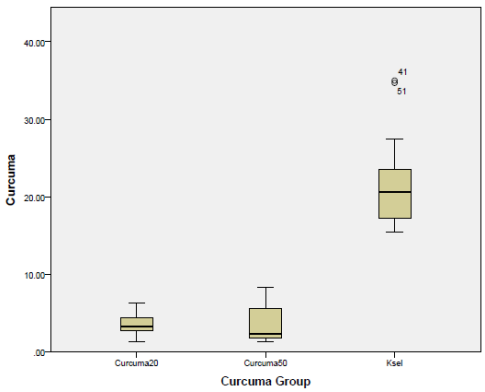

Figure 1: Comparison of the Effects of the Treatment Group on the Control

DISCUSSION

Valve Interstitial Cell Marker Expression and Isolation

VIC obtained from the rabbit valve *O. cuniculus* were employed in this investigation. Comparatively speaking to VIC that weren't TGF-1 exposed, When cells were exposed to TGF-1 cytokines, they differentiated into myofibroblasts, which were distinguished by a noticeably increased expression of SMA. TGF-1 can trigger the transcription of myofibroblastic pro-differentiation genes by activating the Smad transduction pathway.[22,23] Both *in-vitro* and *in-vivo* studies have shown that TGF-1 serves as a strong profibrogenic cytokine,[20, 24-26] have fibrosis, as well as elevated TGF-1 levels in the heart organ.[27-29] The following ways by which TGF-1 exposure induces ventricular fibroblast cells to trigger the fibrosis process were also demonstrated by research by Petrov *et al.* inducing fibroblasts to produce collagen, inducing myofibroblasts to produce collagen, and inducing fibroblasts to differentiate into myofibroblasts.[5] No study hasn't been done yet that uses rabbits to produce *in-vitro* fibrosis models after just one TGF-1 treatment. Regarding the valve isolation technique that Lin *et al.* explained Lin *et al.,* (2017) We changed the way we chose the test animals. The approach presented by Lin *et al*., (2017) has technical issues when choosing mice to use as models., this is due to the small size of the rat heart as well as the valve leaf and valvular apparatus so the valve isolation process has the potential to contamination of fibroblast cells from the atria and ventricles can result in false positive results from isolation of VIC. The success of valve isolation using the method described in chapter 3 is proven by cell morphology on a phase contrast microscope which can be seen in a spindle-shaped pattern and positive luminescence of vimentin markers as a marker of VIC. Through the careful selection of experimental animals, our work was able to develop valvular interstitial cell isolates in a manner similar to that described by Lin *et al*., (2017) with a decreased risk of contamination of ventricular or atrial myocytes and fibroblasts. Activated VIC is a type of valve interstitial cell isolated based on the number of a VIC in the population which is the main resident in normal valves.[30]

Expression of SMA Marker Myofibroblast

One of the key profibrotic cytokines that is heavily implicated in the pathophysiology of rheumatic heart disease is transforming growth factor-1. This cytokine can promote myofibroblastic differentiation, functionally increasing smooth muscle actin (SMA) upregulation, creating growth factors and inflammatory mediators, and boosting the release of collagen I, III, and fibronectin from the extracellular matrix. The activation of TGF-1 initiates a signaling cascade that involves the cell membrane receptor and intracellular proteins Smad 2/3, the mitogen-activated protein kinase (MAPK) pathway, and the

c-Jun N-terminal kinase (JNK) pathway.[15,31] Myofibroblasts are cells with a characteristic twice the size of VIC with an irregular cell membrane and an active endoplasmic reticulum. Electron microscope examination identified myofibroblasts as having a high exocytic vesicle content, uneven cell membranes, and stress fibers in the cytoplasm.[6,32] After differentiation into myofibroblasts, myofibroblast cells contain actin stress fibers due to the upregulation of SMA in their cytoplasm. The presence of these stress fibers gives rise to contractile properties in myofibroblasts.[33,34] The synthesis of SMA in myofibroblasts involves the p38-MK2-HSP27 pathway. P38 is a stress-activated kinase that responds of them to TGF-β1 stimulation. After activation of p38 phosphorylated MK2 (MAPK-Activated Protein Kinase-2), which then sequentially phosphorylated heat shock protein 27 (HSP27). After phosphorylation, the inhibition of actin polymerization by HSP27 will stop, resulting in stabilization and an increase in actin stress fiber production.[35,36] This work demonstrates that following exposure to TGF-1, VIC undergo a process of differentiation into myofibroblasts. These changes were analyzed using a phase contrast microscope which showed large cell morphology, irregular cell membranes, and cytoplasm filled with stress fibers. The characterization of these cells was confirmed by immunocytochemistry by the presence of SMA luminescence.

Quantity Analysis of Expression of SMA Myofibroblasts

After being exposed to pathological insult, VIC differentiate as a physiological reaction. Then, this damage triggers the production of TGF-1 and starts the transcription of smooth muscle actin. The capacity of myofibroblasts to make collagen and adhesion molecules (tensin, paxilin, and Fibronectin Domain ED-A) will rise.[37,38] Myofibroblasts can exert tension on histological tissue structures because they produce a lot of microfilaments and extracellular fibronectin.[38] By increasing the distance between functional cells (VIC), cell-cell adhesion connections are broken (Connexin 43 and Connex 45), and causing tissue contraction, the transformation of VIC into myofibroblasts would compromise valve function at the macroscopic level.[32] SMA expression is one of the parameters of interstitial cell differentiation into myofibroblasts. This myofibroblastic differentiation functionally will increase the ability to produce inflammatory mediators, growth factors, collagen, and fibronectin secretion up to 10 times and amplify the fibrogenesis process. In several organs, including the heart, lungs, and liver, myofibroblastic differentiation followed by a rise in fibrotic disease has been seen.[33,39] This study demonstrates that TGF-1 was chosen successfully to induce the development of VIC. Changes in cell shape, including changes in size and the presence of stress fibers in the cytoplasm, show that the cells that we described utilizing TGF-1 transformed VIC into myofibroblasts. A substantial amount of SMA fluorescence was seen compared to the TGF-1-unexposed control group of VIC., which became another parameter for selecting TGF-β1 as an inductor agent in this study. We have not found any *in-vitro* studies that induce VIC using TGF-1 has historically induced myofibroblast differentiation. A previous study by Zhou J *et al.* carried out the induction of porcine valve interstitial cell differentiation using interleukin-18.[40] Research by Latif N *et al.* showed the success of induction using fibroblast growth factor.[41] Another study by Ma H *et al.* and Hjortnaes J *et al.* carried out the modulation of matrix elasticity and the use of hydrogel culture media as an inductor of differentiation of VIC into myofibroblasts.[42 43,44]

Effect of Curcumin on Inhibition of Myofibroblastic Differentiation based on SMA Expression

Turmeric has been known to have about 80 species distributed throughout tropical Asia from India, South China, Southeast Asia, Papua New Guinea, and Northern Australia.[45] The anti-inflammatory activity of curcumin is significant in both acute and chronic inflammatory models. Curcumin is safe in several trials and has demonstrated anti-inflammatory properties.[46] The key process in initiating heart valve fibrosis, characterized by the expression of -SMA and the deposition of structural components of the ECM and collagen such as collagen types I and III, is the differentiation of fibroblasts into myofibroblasts. Nuclear factor (NF)-kB has been known to be one of the cytokines that play a role in the inflammatory process which is also activated by most inflammatory cytokines, one of which is a gram bacterial infection. Curcumin appears to have the ability to inhibit the activation of NF-B which is increased by several different inflammatory stimuli by inhibiting the activation of tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1). By suppressing inflammation through many mechanisms, curcumin has been shown to prevent the onset of the fibrosis process.[47] Curcumin also has involvement in several mechanisms that inhibit cytokine production. Several targets of curcumin, namely suppressing transcription factors other than NF-kB can also activate protein-1 on sensitized macrophages and monocytes, which will cause inhibition of cytokine gene expression.[48] As a strong pro-fibrotic factor in the cardiovascular system, angiotensin II has an important role in the process of fibroblast proliferation and can induce cardiac collagen synthesis *in-vitro* and *in-vivo.*[49] Curcumin significantly reduces the expression of collagen types I and III induced by Ang II and in heart valve fibroblasts. The intervention of heart valve fibroblasts with curcumin effectively inhibited Ang II-induced TGF-β1 activation. The activity of MMP-9 as the driving force behind the degradation of the extracellular matrix was attenuated by the administration of curcumin. Furthermore, curcumin was able to suppress the intracellular generation of TIMP-1 expression induced by Ang II.[44] Extracellular signaling-related kinase (ERK) is a key member of the mitogen-activated protein kinase class, which is closely associated with Ang II-induced cardiomyocyte remodeling. Acting as a cytokine with another pathway that is closely related to cardiomyocyte remodeling in this case the formation of fibroblasts in myocyte cells induced by Ang II, JAK/STAT has a different pathway from that activated by mitogen-activated protein kinases. In several studies, the JAK/STAT signaling pathway has played a central role in cardiac

pathophysiology. In addition, JAK/STAT signaling represents a branch of the autocrine loop for the Ang II pathway, which amplifies Ang II activity in cardiomyocytes.[50] This could explain the process of inhibition of curcumin in the JAK/STAT and ERK pathways induced by Ang II. We found that pretreatment with curcumin significantly inhibited the expression of a-SMA protein induced by TGF 1.

CONCLUSION

TGF-1 induces interstitial cells in the rabbit valve to differentiate into myofibroblasts based on the expression of smooth muscle (20 nanoM/L). There was an inhibitory effect of high doses of curcumin (50 nanoM/L) on the differentiation of TGF-β1-induced rabbit. Based on the expression of smooth muscle actin in comparison to controls, interstitial cells are

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REFERENCES

1. Aluru JS, Barsouk A, Saginala K, Rawla P, Barsouk A. Valvular Heart Disease Epidemiology. Medical Sciences. 2022 Jun 15;10(2):32.
2. Aikawa E, Whittaker P, Farber M, Mendelson K, Padera RF, Aikawa M, Schoen FJ. Human semilunar cardiac valve remodeling by activated cells from fetus to adult: implications for postnatal adaptation, pathology, and tissue engineering. Circulation. 2006 Mar 14;113(10):1344-52.
3. Hocevar BA, Howe PH. Analysis of TGFβ-mediated synthesis of extracellular matrix components. Transforming Growth Factor-Beta Protocols. 2000:55-65.
4. Lis YM, Burleigh MC, Parker DJ, Child AH, Hogg J, Davies MJ. Biochemical characterization of individual normal, floppy and rheumatic human mitral valves. Biochemical Journal. 1987 Jun 15;244(3):597-603.
5. Lijnen PJ, Petrov VV, Fagard RH. Induction of cardiac fibrosis by transforming growth factor-β1. Molecular genetics and metabolism. 2000 Sep 1;71(1-2):418-35.
6. Patrov VV, Fagard RH, Lijnen PJ. Stimulation of collagen production by transforming growth factor-h1 during differentiation of cardiac fibroblasts to myofibroblasts. Hypertension. 2002;39(2):258-63.
7. Walker GA, Masters KS, Shah DN, Anseth KS, Leinwand LA. Valvular myofibroblast activation by transforming growth factor-β: implications for pathological extracellular matrix remodeling in heart valve disease. Circulation research. 2004 Aug 6;95(3):253-60.
8. Ogata H, Noguch H, Ohtsubo T, Liao J, Kohara H, Yamada K. Angiotensin II type I receptor blocker, Losartan, inhibits fibrosis in liver by suppressing TGF-beta1 production. Integr Mol Med. 2016;3(1):520-3.
9. Attisano L, Wrana JL. Signal transduction by the TGF-β superfamily. Science. 2002 May 31;296(5573):1646-7.
10. Pardali E, Ten Dijke P. TGFβ signaling and cardiovascular diseases. International journal of biological sciences. 2012;8(2):195.
11. Vaideeswar P, Butany J. Valvular Heart Disease. Ed ke-4. Elsevier Inc. 2016.
12. World Health Organization. Rheumatic Fever and Rheumatic Heart Disease: Report of a WHO expert Consultation, Geneva, 29 October-1 November, 2001. World Health Organization; 2004 Feb 4.
13. Kumar RK, Antunes MJ, Beaton A, Mirabel M, Nkomo VT, Okello E, Regmi PR, Reményi B, Sliwa-Hähnle K, Zühlke LJ, Sable C. Contemporary diagnosis and management of rheumatic heart disease: implications for closing the gap: a scientific statement from the American Heart Association. Circulation. 2020 Nov 17;142(20):e337-57.
14. Rutkovskiy A, Malashicheva A, Sullivan G, Bogdanova M, Kostareva A, Stensløkken KO, Fiane A, Vaage J. Valve interstitial cells: the key to understanding the pathophysiology of heart valve calcification. Journal of the American Heart Association. 2017 Sep 14;6(9):e006339.
15. Du Y, Xiao H, Wan J, Wang X, Li T, Zheng S, Feng J, Ye Q, Li J, Li G, Fan Z. Atorvastatin attenuates TGF-β1-induced fibrogenesis by inhibiting Smad3 and MAPK signaling in human ventricular fibroblasts. International Journal of Molecular Medicine. 2020 Aug 1;46(2):633-40.
16. Dorai T, Diouri J, O'Shea O, Doty SB. Curcumin inhibits prostate cancer bone metastasis by up-regulating bone morphogenic protein-7 in vivo. Journal of cancer therapy. 2014 Apr 4;5(4):369.
17. Chen X, Chen X, Shi X, Gao Z, Guo Z. Curcumin attenuates endothelial cell fibrosis through inhibiting endothelial–interstitial transformation. Clinical and Experimental Pharmacology and Physiology. 2020 Jul;47(7):1182-92.
18. Masuda A, Nakamura T, Abe M, Iwamoto H, Sakaue T, Tanaka T, Suzuki H, Koga H, Torimura T. Promotion of liver regeneration and anti-fibrotic effects of the TGF-β receptor kinase inhibitor galunisertib in CCl4-treated mice. International journal of molecular medicine. 2020 Jul 1;46(1):427-38.
19. Guzy R. Fibroblast growth factor inhibitors in lung fibrosis: friends or foes?. American Journal of Respiratory Cell and Molecular Biology. 2020 Sep;63(3):273-4.
20. Peng H, Carretero OA, Peterson EL, Rhaleb NE. Ac-SDKP inhibits transforming growth factor-β1-induced differentiation of human cardiac fibroblasts into myofibroblasts. American Journal of Physiology-Heart and Circulatory Physiology. 2010 May;298(5):H1357-64.
21. Zhou J, Jiang K, Ding X, Fu M, Wang S, Zhu L, He T, Wang J, Sun A, Hu K, Chen L. Qiliqiangxin inhibits angiotensin II-induced transdifferentiation of rat cardiac fibroblasts through suppressing interleukin-6. Journal of cellular and molecular medicine. 2015 May;19(5):1114-21.
22. Schimierer B, Hill CS. TGFbeta-SMAD signal transduction: Molecular specificity and functional flexibitily. Nat. Rev. Mol. Cell Biol. 2007;8:970-82.
23. Shannon TR, Guo T, Bers DM. Ca2+ scraps: local depletions of free [Ca2+] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca2+ reserve. Circulation research. 2003 Jul 11;93(1):40-5.
24. Calderone A, Murphy RJ, Lavoie J, Colombo F, Béliveau L. TGF-β1 and prepro-ANP mRNAs are differentially regulated

in exercise-induced cardiac hypertrophy. Journal of Applied Physiology. 2001 Aug 1;91(2):771-6.
25. Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, Thannickal VJ, Cardoso WV, Lü J. miR-29 is a major regulator of genes associated with pulmonary fibrosis. American journal of respiratory cell and molecular biology. 2011 Aug;45(2):287-94.
26. Frangogiannis NG. Transforming growth factor–β in tissue fibrosis. Journal of Experimental Medicine. 2020 Mar 2;217(3).
27. Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K, Iwao H. Angiotensin II induces cardiac phenotypic modulation and remodeling in vivo in rats. Hypertension. 1995 Jun;25(6):1252-9.
28. Kupfahl C, Pink D, Friedrich K, Zurbrügg HR, Neuss M, Warnecke C, Fielitz J, Graf K, Fleck E, Regitz-Zagrosek V. Angiotensin II directly increases transforming growth factor β1 and osteopontin and indirectly affects collagen mRNA expression in the human heart. Cardiovascular research. 2000 Jun 1;46(3):463-75.
29. Sun Y, Zhang JQ, Zhang J, Lamparter S. Cardiac remodeling by fibrous tissue after infarction in rats. Journal of Laboratory and Clinical Medicine. 2000 Apr 1;135(4):316-23.
30. Lin C, Zhu D, Markby G, Corcoran BM, Farquharson C, Macrae VE. Isolation and characterization of primary rat valve interstitial cells: A new model to study aortic valve calcification. JoVE (Journal of Visualized Experiments). 2017 Nov 20(129):e56126.
31. Heldin CH, Miyazono K, Ten Dijke P. TGF-β signalling from cell membrane to nucleus through SMAD proteins. Nature. 1997 Dec;390(6659):465-71.
32. Baum J, Duffy HS. CityPaws Animal Hospital 1823. J. Cardiovasc. Pharmacol. 2011;57(4):376-9.
33. Hinz B, Gabbiani G. Mechanisms of force generation and transmission by myofibroblasts. Current opinion in biotechnology. 2003 Oct 1;14(5):538-46.
34. Sousa AM, Liu T, Guevara O, Stevens J, Fanburg BL, Gaestel M, Toksoz D, Kayyali US. Smooth muscle α-actin expression and myofibroblast differentiation by TGFβ are dependent upon MK2. Journal of cellular biochemistry. 2007 Apr 15;100(6):1581-92.
35. Kayyali US. Pennella CM, Trujillo C, Villa O, Gaestel M, and Hassoun PM. Cytoskeletal changes in hypoxic pulmonary endothelial cells are dependent on MAPK-activated protein kinase MK2. J Biol Chem. 2002;277:42596-602.
36. An SS, Pennella CM, Gonnabathula A, Chen J, Wang N, Gaestel M, Hassoun PM, Fredberg JJ, Kayyali US. Hypoxia alters biophysical properties of endothelial cells via p38 MAPK- and Rho kinase-dependent pathways. American Journal of Physiology-Cell Physiology. 2005 Sep;289(3):C521-30.
37. Frangogiannis NG, Michael LH, Entman ML. Myofibroblasts in reperfused myocardial infarcts express the embryonic form of smooth muscle myosin heavy chain (SMemb). Cardiovascular research. 2000 Oct 1;48(1):89-100.
38. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2003 Jul;200(4):500-3.
39. Chen M, Li H, Wang G, Shen X, Zhao S, Su W. Atorvastatin prevents advanced glycation end products (AGEs)-induced cardiac fibrosis via activating peroxisome proliferator-activated receptor gamma (PPAR-γ). Metabolism. 2016 Apr 1;65(4):441-53.
40. Zhou J, Zhu J, Jiang L, Zhang B, Zhu D, Wu Y. Interleukin 18 promotes myofibroblast activation of valvular interstitial cells. International journal of cardiology. 2016 Oct 15;221:998-1003.
41. Latif N, Quillon A, Sarathchandra P, McCormack A, Lozanoski A, Yacoub MH, Chester AH. Modulation of human valve interstitial cell phenotype and function using a fibroblast growth factor 2 formulation. PLoS One. 2015 Jun 4;10(6):e0127844.
42. Ma H, Killaars AR, DelRio FW, Yang C, Anseth KS. Myofibroblastic activation of valvular interstitial cells is modulated by spatial variations in matrix elasticity and its organization. Biomaterials. 2017 Jul 1;131:131-44.
43. Hjortnaes J, Camci-Unal G, Hutcheson JD, Jung SM, Schoen FJ, Kluin J, Aikawa E, Khademhosseini A. Directing valvular interstitial cell myofibroblast-like differentiation in a hybrid hydrogel platform. Advanced healthcare materials. 2015 Jan;4(1):121-30.
44. Mao Y, Tokudome T, Kishimoto I. Paper Cardboard Toothpaste Box Packaging and Printing. Journal of Geriatric Cardiology: JGC. 2014 Sep;11(3):253.
45. Sirirugsa P, Larsen K, Maknoi C. The genus Curcuma L.(Zingiberaceae): distribution and classification with reference to species diversity in Thailand. Gard Bull Sing. 2007;59(2):203-20.
46. Rathore S, Mukim M, Sharma P, Devi S, Nagar JC, Khalid M. Curcumin: A review for health benefits. Int. J. Res. Rev. 2020;7(1):273-90.
47. Panahi Y, Hosseini MS, Khalili N, Naimi E, Simental-Mendía LE, Majeed M, Sahebkar A. Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial. Biomedicine & pharmacotherapy. 2016 Aug 1;82:578-82.
48. Shehzad A, Khan S, Sup Lee Y. Curcumin molecular targets in obesity and obesity-related cancers. Future Oncology. 2012 Feb;8(2):179-90.
49. Zhou L, Shao Y, Huang Y, Yao T, Lu LM. 17β-Estradiol inhibits angiotensin II-induced collagen synthesis of cultured rat cardiac fibroblasts via modulating angiotensin II receptors. European journal of pharmacology. 2007 Jul 19;567(3):186-92.
50. Fisher M, Liu B, Glennon PE, Southgate KM, Sale EM, Sale GJ, Lewis MJ, Groves PH. Downregulation of the ERK 1 and 2 mitogen activated protein kinases using antisense oligonucleotides inhibits proliferation of porcine vascular smooth muscle cells. Atherosclerosis. 2001 Jun 1;156(2):289-95.