

Review Article

Genetically Modified Mouse Models Used for Studying the Role of the AT₂ Receptor in Cardiac Hypertrophy and Heart Failure

Maria D. Avila,¹ James P. Morgan,² and Xinhua Yan²

¹Department of Internal Medicine, Carney Hospital, Tufts University School of Medicine, Boston, MA 02124, USA

²Department of Cardiovascular Research, St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, MA 02135, USA

Correspondence should be addressed to Xinhua Yan, xinhua.yan@tufts.edu

Received 18 September 2010; Revised 15 February 2011; Accepted 21 February 2011

Academic Editor: Oreste Gualillo

Copyright © 2011 Maria D. Avila et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The actions of Angiotensin II have been implicated in many cardiovascular conditions. It is widely accepted that the cardiovascular effects of Angiotensin II are mediated by different subtypes of receptors: AT₁ and AT₂. These membrane-bound receptors share a part of their nucleic acid but seem to have different distribution and pathophysiological actions. AT₁ mediates most of the Angiotensin II actions since it is ubiquitously expressed in the cardiovascular system of the normal adult. Moreover AT₂ is highly expressed in the developing fetus but its expression in the cardiovascular system is low and declines after birth. However the expression of AT₂ appears to be modulated by pathological states such as hypertension, myocardial infarction or any pathology associated to tissue remodeling or inflammation. The specific role of this receptor is still unclear and different studies involving *in vivo* and *in vitro* experiments have shown conflicting data. It is essential to clarify the role of the AT₂ receptor in the different pathological states as it is a potential site for an effective therapeutic regimen that targets the Angiotensin II system. We will review the different genetically modified mouse models used to study the AT₂ receptor and its association with cardiac hypertrophy and heart failure.

1. Introduction

Angiotensin II (Ang II) plays a pivotal role in the regulation of the cardiovascular system [1, 2]. It is known that the effects of Ang II are mediated by several subtypes of Ang II receptors; these receptor subtypes differ from each other on their pharmacological and biochemical properties. Up until now, the subtypes that have been identified are the Type 1 (AT₁) and the Type 2 (AT₂) present in humans, and AT_{1A} and AT_{1B} present in rodents [2]. Both of these receptors, AT₁ and AT₂, belong to the seven-transmembrane domain superfamily of receptors, and they share 34% of their nucleic acid sequence. The AT₁ receptor is widely distributed in adult tissues (blood vessels, kidney, adrenal gland, heart liver, and brain). The AT₂ receptor is ubiquitously expressed in fetal tissues but the expression of the AT₂ receptor declines rapidly after birth with very low abundance in ventricular myocytes and vascular endothelium and absence in fibroblasts [3, 4]. Most of the distinct effects of Ang II on vasomotor tone,

contractility, and myocardial growth are mediated by AT₁ receptors [5, 6] but various studies from the past two decades have demonstrated involvement of the AT₂ receptor in some important actions of Ang II in the heart. However, the specific role of the AT₂ receptor still remains unclear [5–7] as there have been contradictory results in the different studies performed.

Accumulating evidence suggests that the AT₂ receptor may act as an AT₁ receptor antagonist, as its activation has been associated with opposite cellular functions of AT₁, such as antigrowth, antihypertrophic, and proapoptotic effects [8, 9]. If these receptors exert opposing actions in the heart, their ratio of expression under different cardiac pathologies may determine myocardial function and structure. Experiments using hypertrophic hearts have demonstrated that the cardiac expression of AT₁ and AT₂ receptors changes during the process of cardiac hypertrophy allowing the heart to respond differently to Ang II. Studies have shown that the pathological hypertrophy and failure of human hearts

are associated with a decrease of AT₁ and an increase of AT₂ receptor expression, that is, an increase of AT₂-to-AT₁ ratio [10, 11]. In patients treated with AT₁ antagonists, circulating Ang II levels are increased and may preferentially bind to AT₂ receptors inducing several effects that still remain controversial.

In order to develop a safer and more effective therapeutic regimen by targeting the Ang II system, it becomes essential to clarify the role of the AT₂ receptor in the development of cardiac hypertrophy and failure. With the objective of clarifying the specific actions of the AT₂ receptor in cardiac hypertrophy and heart failure, different approaches have been developed including *in vivo* and *in vitro* experiments. However, results from these studies have been inconsistent. This paper will review the different genetically modified mouse models used to study the AT₂ receptor and its association with cardiac hypertrophy and heart failure.

2. Angiotensin II Type 2 Receptor Mouse Models

Mice have been extensively used as a model for cardiovascular research; not only due to their short gestation period but also because there is significant preservation of the molecular pathways that control cardiovascular development and function between mice and humans [12]. Different approaches to genetic modification in the mouse such as gene deletion or overexpression have been described [13]. These animal models have become invaluable tools to study cardiovascular genetics, developmental biology, and physiology in normal or pathologic hearts [12, 13].

Compared to *in vitro* cardiomyocyte culture and pharmacological intervention, genetically modified mouse models have provided a novel and powerful method to study the physiological function of the AT₂ receptor. First, this technique allows us to study the function of the gene of interest in a physiological setting; second, it reduces the off-target effects of pharmacological inhibitors. It was hoped that genetic deletion or overexpression of the receptor would provide a much clearer picture of AT₂ in cardiac hypertrophy and failure. Currently, there are two AT₂ overexpression and two AT₂ knockout mouse models that have been generated [14–17]. Unexpectedly, the results from these mouse models are contradictory and have raised more questions in the field.

2.1. Transgenic Mice with Cardiomyocyte-Specific Overexpression of AT₂. Two transgenic (TG) mouse models with cardiomyocyte-specific AT₂ overexpression have been generated [14, 15]. In the first model, the AT₂ receptor was overexpressed in both atria and ventricles, using the α -myosin heavy chain promoter in C57BL/6 mice [15]. Studies using this transgenic mouse model showed that the TG mice did not present any abnormality in myocardial development or phenotype when compared to nontransgenic (NTG) mice [15, 18]. Under baseline condition, the heart weight (HW) to body weight (BW) ratio was similar between TG and NTG mice. However, TG mice showed a higher end-diastolic wall thickness [18, 19]. Heart rate was similar between TG and NTG, while ejection fraction (EF%) was higher in TG mice

[18]. Aortic stenosis (AS) in adult mice or with chronic Ang II infusion significantly increased HW/BW in mice compared to control mice; but HW/BW was not different between TG and NTG mice [19]. Ang II infusion in mice reduced HR in TG mice, but did not increase apoptosis [18]. Myocardial infarction (MI) increased left ventricular mass index (LVMI) in mice, with no difference between TG and NTG mice. LV wall thickness and EF%, however, maintained higher in TG versus NTG mice after MI [18] (Table 1).

Our laboratory has generated a mouse model with ventricular myocyte-specific overexpression of the AT₂ receptor using α -myosin heavy chain 2v (MLC 2v) promoter in FVB/n mice [14]. We generated four lines of mice with different copy number of the AT₂ gene [14]. This allowed us to study the dose-response of AT₂ overexpression. We have studied two lines of AT₂ transgenic mice with relatively high (AT₂^{high}TG) and low (AT₂^{low}TG) expression of AT₂. We found that under baseline condition, the left ventricular to body weight ratio (LV/BW) was increased in AT₂^{high}TG mice; this was accompanied by a decrease of LV wall thickness, an increase of cardiomyocyte area and length, and an increase of interstitial spaces and the deposition of fibrillar collagen in AT₂^{high}TG mice [14]. LV systolic function, as assessed by echocardiography and hemodynamic measurements, was significantly depressed in AT₂^{high}TG [14]. The contractile function of cardiomyocytes isolated from AT₂^{high}TG mice was significantly decreased under baseline and in response to Ang II [20]. These results suggest that excessive AT₂ overexpression can induce pathological cardiac remodeling and failure. Mice with low AT₂ overexpression (AT₂^{low}TG), however, did not demonstrate a significant change in cardiac morphology and function at baseline [14]. We further tested whether AT₂ overexpression would modify cardiac remodeling in aortic stenosis- (AS-) induced hypertrophy using AT₂^{low}TG mice [21]. Our results showed that 70 days after AS, LV/BW and LV wall thickness were increased in AS mice, with no difference between AT₂^{low}TGAS and NTGAS mice. However, LV myocyte diameter was smaller and the percentage of LV collagen was lower in AT₂^{low}TGAS versus NTGAS mice. LV systolic pressure and peak dP/dt± were lower in AT₂^{low}TGAS versus NTGAS mice, with no decrease in wall thickness. LV end diastolic pressure was lower in AT₂^{low}TGAS versus NTGAS mice [21]. These results suggest that lower level AT₂ overexpression did not accelerate cardiac hypertrophy and failure in AS mice; it is likely that the diastolic compliance was improved in AT₂^{low}TGAS mice (Table 1).

These two transgenic mouse models are different in several aspects: (1) the strain of mice (C57BL/6 versus FVB/n), (2) the AT₂ overexpression site (atria + ventricles versus ventricles), and (3) the overexpression level of AT₂. The site of AT₂ overexpression may be the cause of HR changes in mice using α -MHC promoter [15], which was not observed in mice using MLC2v promoter [14]. Despite the difference of the models, the results from these studies demonstrate that the expression level of AT₂ is a key determinant of outcome. Excessive AT₂ overexpression can lead to cardiac failure, while lower AT₂ overexpression may improve cardiac performance under stress.

2.2. AT₂ Knockout Mouse Models. Two AT₂ knockout (KO) mouse models were generated at the same time by two independent research groups [16, 17]. Both models were generated by targeted disruption of the AT₂ gene on the X chromosome [16, 17]. The results from these two models, however, are different. The first model was generated in C57BL/6 mice [16]. Under baseline condition, blood pressure (BP) was higher in KO mice. LVW/BW, wall thickness and LV mass (LVM) were lower in KO versus wild-type (WT) mice [16, 22, 23]. Cardiac function did not change in KO versus WT [22]. The most striking result from this model is that AT₂ knockout prevented the cardiac hypertrophic response to both aortic stenosis (AS) and chronic Ang II infusion in mice [22, 23]. Cardiac function was either similar between KO and WT (AS) or improved in KO versus WT (Ang II infusion) mice [22, 23]. These results suggest that AT₂ is essential for the development of cardiac hypertrophy and dysfunction (Table 1).

The second AT₂ KO mouse model was generated in FVB/n mice [17]. Unlike the first model, under baseline condition, BP was not different between KO and WT and no changes in cardiac morphology were observed [24]. Aortic stenosis resulted in a similar increase of HW/BW in KO versus WT mice, while the perivascular fibrosis was higher in KO mice [24]. Acute myocardial infarction resulted in higher mortality rate, higher LVW/BW, lung/BW, ratio and decreased EF% in KO versus WT mice [25] (Table 1). These results suggest that AT₂ has antihypertrophic remodeling effects and may be important for maintaining cardiac function under certain stress.

The contradictory results from these two models may be caused by the different mouse strains and the different disease models used. However, they also suggest that traditional gene deletion approach in mice may lead to the activation of compensatory mechanisms and ultimately different phenotypes.

3. Limitations of the Existing Mouse Models

The existing AT₂ mouse models have several limitations: (1) the models cannot recapitulate the AT₂ receptor expression patterns during pathological hypertrophy and failure. Studies have shown that AT₂ receptor expression is high in the fetus [3], significantly decreased in adult hearts, and increased again in diseased hearts in humans [7]. While AT₂ receptors are chronically overexpressed or disrupted in these models; (2) the expression level of AT₂ receptors in transgenic mouse hearts may not represent the increase of AT₂ receptors in a diseased heart. Studies in transgenic mice clearly showed a dose-relationship between AT₂ overexpression and cardiac remodeling and function. Further experiments by using mice with cardiac AT₂ receptors expression similar to that in diseased hearts are needed; (3) the cell type of AT₂ receptor overexpression may be not accurate. Studies have shown that fibroblasts are the major cell type that expresses AT₂ receptors in diseased human hearts [26]. In current mouse models the AT₂ receptor is overexpressed in cardiomyocytes; (4) chronically

manipulation of AT₂ receptors expression may activate compensatory mechanisms, which may lead to phenotypes that are not related to AT₂ receptors.

3.1. In Vitro Studies. In order to understand better the role of AT₂ receptors in the heart and in cardiac pathology, it is important to review what *in vitro* studies have shown and how they differ from the mouse models mentioned above. Studies using cultured rat neonatal cardiomyocytes, fibroblasts, and coronary endothelial cells have shown that the stimulation of the AT₂ receptor inhibits cell growth and proliferation and opposes the effects of the AT₁ receptor [27, 28]. Nakajima C. et al. used AT₂ receptor expression vectors to evaluate the growth of cultured aortic vascular smooth muscle cells (VSMC) with overexpression of these receptors versus controls. In this study, VSMCs with transfection of the AT₂ receptor presented a decrease of 70% in neointimal area when compared to controls, suggesting that the AT₂ receptors have an inhibitory effect of neointimal growth. Moreover, this effect was blocked with PD123319, an AT₂ receptor antagonist [29].

On the other hand, a direct prohypertrophic action of AT₂ receptors on cardiomyocytes was demonstrated by D'Amore et al. when using adenoviruses encoding AT₁ and AT₂ to coexpress these receptors in isolated cardiomyocytes [30]. Overexpression of the AT₂ receptor on cardiomyocytes using adenoviruses provoked an increase in the basal hypertrophy of these cells. This was unaffected by Ang II or AT₂ receptor ligands such as PD123319 or CGP42112A. The major outcome of this study was the lack of evidence to demonstrate that the AT₂ receptor opposes the actions of the AT₁ receptor, a widely proposed view. When the expression of the AT₂ receptor was increased, the Ang II-mediated hypertrophy through the AT₁ receptor was not inhibited; moreover, the AT₂ receptor-mediated enhanced basal hypertrophy was unchanged and it was added to that of the AT₁ receptor. These findings suggest that the AT₁ and AT₂ receptor might use different pathways.

Results from *in vitro* cell culture have provided invaluable information regarding the role of the AT₂ receptor in mediating the Ang II signaling and the interaction of the AT₁ and AT₂ receptor in specific cell types. Different studies involving the AT₂ receptors showed that there is marked tissue heterogeneity, likely a reflection of the balance of AT₁/AT₂ receptor expression [31]. The various growth effects of Ang II seen in the *in vitro* studies were determined by the type of AT₂ receptor expressed in the cultured cell. For example, the AT₂ receptors are constitutively expressed in cultured endothelial cells but not in cultured vascular smooth muscle cells (VSMC); consequently, the AT₂ receptor antiproliferative effects will counteract the AT₁ receptor growth promoting effects in endothelial cells but not in vascular smooth muscle cells [27, 29]. This might explain why the results of the different *in vitro* studies are not 100% consistent and why these results differ from *in vivo* experiments. Furthermore, cell culture may not reflect the complex cross-talk among different cell types in the heart *in vivo*. In regards to the studies of the diseased heart even

TABLE 1: Cardiac phenotype and function in mice with AT₂ overexpression (TG) or knock out (KO).

Mouse model	Strain	Baseline	Disease state	References
AT ₂ TG mice (cardiomyocyte-specific, α -MHC)	C57BL/6	\leftrightarrow HW/BW ↑PW ↑EF%	AS: \leftrightarrow HW/BW Ang II: \leftrightarrow HW/BW, ↓HR MI: \leftrightarrow LVMI, ↑PW, ↑EF%	[15, 18, 19]
AT ₂ ^{high} TG		↑LVW/BW, ↓wall thickness ↑interstitial collagen ↑myocyte area and length		[14]
AT ₂ TG mice (ventricular myocyte-specific, MLC-2v)	FVB/n	↓LV contractile function ↑apoptosis	AS: \leftrightarrow LVW/BW \leftrightarrow wall thickness ↓myocyte diameter	
AT ₂ ^{low} TG		\leftrightarrow cardiac morphology and function	↓interstitial collagen ↓LVSP ↓LVEDP	[21]
AT ₂ KO mice	C57BL/6	↑BP ↑HR ↓LVW/BW ↓wall thickness ↓LVMI \leftrightarrow contractile function	AS: No hypertrophy ↓interstitial collagen \leftrightarrow cardiac function Ang II: No hypertrophy \leftrightarrow BP ↓interstitial collagen ↑diastolic function	[16, 22, 23]
AT ₂ KO mice	FVB/n	\leftrightarrow BP \leftrightarrow cardiac morphology	AS: \leftrightarrow hypertrophy ↑perivascular fibrosis ↑coronary arterial thickening AMI: ↑LVW/BW ↑Lung/BW ↓EF%	[17, 24, 25]

HW: heart weight; LVW: left ventricular weight; BP: blood pressure; HR: heart rate; LVMI: left ventricular mass index; PW: posterior wall thickness; EF: ejection fraction; AS: aortic stenosis; Ang II: Ang II infusion; MI: myocardial infarction.

though it is well known that AT₂ receptors are upregulated in cardiac fibroblasts in the presence of cardiac pathology [26, 32, 33], it is not known whether the ratio of AT₁/AT₂ by overexpression of these receptors in cell cultures represented that in a hypertrophied/failing heart.

4. Conclusions

Transgenic mouse models with specific AT₂ overexpression or disruption have provided new information on this receptor. However, these results need to be interpreted with caution. New transgenic mouse models that conditionally overexpress or disrupt AT₂ in specific cell types in addition to cardiomyocytes in the heart may be used for more precisely studying the pathophysiological role of AT₂ receptors.

Acknowledgment

This work is supported by the American Heart Association Grant-In-Aid (Yan X, 10GRNT4710003).

References

- [1] B. H. Lorell, "Role of angiotensin AT₁ and AT₂ receptors in cardiac hypertrophy and disease," *American Journal of Cardiology*, vol. 83, no. 12A, pp. 48H–52H, 1999.
- [2] H. Matsubara, "Renin-angiotensin system in human failing hearts: message from nonmyocyte cells to myocytes," *Circulation Research*, vol. 88, no. 9, pp. 861–863, 2001.
- [3] E. F. Grady, L. A. Sechi, C. A. Griffin, M. Schambelan, and J. E. Kalinyak, "Expression of AT₂ receptors in the developing rat fetus," *Journal of Clinical Investigation*, vol. 88, no. 3, pp. 921–933, 1991.

- [4] Z. Q. Wang, A. F. Moore, R. Ozono, H. M. Siragy, and R. M. Carey, "Immunolocalization of subtype 2 angiotensin II (AT₂) receptor protein in rat heart," *Hypertension*, vol. 32, no. 1, pp. 78–83, 1998.
- [5] G. W. Booz and K. M. Baker, "Role of type 1 and type 2 angiotensin receptors in angiotensin II-induced cardiomyocyte hypertrophy," *Hypertension*, vol. 28, no. 4, pp. 635–640, 1996.
- [6] M. D. Schneider and B. H. Lorell, "AT₂, judgment day: which angiotensin receptor is the culprit in cardiac hypertrophy?" *Circulation*, vol. 104, no. 3, pp. 247–248, 2001.
- [7] G. W. Booz, "Cardiac angiotensin AT₂ receptor. What exactly does it do?" *Hypertension*, vol. 43, no. 6, pp. 1162–1163, 2004.
- [8] M. Horiuchi, M. Akishita, and V. J. Dzau, "Recent progress in angiotensin II type 2 receptor research in the cardiovascular system," *Hypertension*, vol. 33, no. 2, pp. 613–621, 1999.
- [9] L. H. Opie and M. N. Sack, "Enhanced angiotensin II activity in heart failure: reevaluation of the counterregulatory hypothesis of receptor subtypes," *Circulation Research*, vol. 88, no. 7, pp. 654–658, 2001.
- [10] K. Asano, D. L. Dutcher, J. D. Port et al., "Selective downregulation of the angiotensin II AT₁-receptor subtype in failing human ventricular myocardium," *Circulation*, vol. 95, no. 5, pp. 1193–1200, 1997.
- [11] V. Regitz-Zagrosek, N. Friedel, A. Heymann et al., "Regulation, chamber localization, and subtype distribution of angiotensin II receptors in human hearts," *Circulation*, vol. 91, no. 5, pp. 1461–1471, 1995.
- [12] T. Senbonmatsu, T. Saito, E. J. Landon et al., "A novel angiotensin II type 2 receptor signaling pathway: possible role in cardiac hypertrophy," *The EMBO Journal*, vol. 22, no. 24, pp. 6471–6482, 2003.
- [13] S. Izumo and T. Shioi, "Cardiac transgenic and gene-targeted mice as models of cardiac hypertrophy and failure: a problem of (new) riches," *Journal of Cardiac Failure*, vol. 4, no. 4, pp. 263–270, 1998.
- [14] X. Yan, R. L. Price, M. Nakayama et al., "Ventricular-specific expression of angiotensin II type 2 receptors causes dilated cardiomyopathy and heart failure in transgenic mice," *American Journal of Physiology*, vol. 285, no. 5, pp. H2179–H2187, 2003.
- [15] H. Masaki, T. Kurihara, A. Yamaki et al., "Cardiac-specific overexpression of angiotensin II AT₂ receptor causes attenuated response to AT₁ receptor-mediated pressor and chronotropic effects," *Journal of Clinical Investigation*, vol. 101, no. 3, pp. 527–535, 1998.
- [16] T. Ichiki, P. A. Labosky, C. Shiota et al., "Effects on blood pressure exploratory behaviour of mice lacking angiotensin II type 2 receptor," *Nature*, vol. 377, no. 6551, pp. 748–750, 1995.
- [17] L. Hein, G. S. Barsh, R. E. Pratt, V. J. Dzau, and B. K. Kobilka, "Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice," *Nature*, vol. 377, no. 6551, pp. 744–747, 1995.
- [18] Z. Yang, C. M. Bove, B. A. French et al., "Angiotensin II type 2 receptor overexpression preserves left ventricular function after myocardial infarction," *Circulation*, vol. 106, no. 1, pp. 106–111, 2002.
- [19] H. Sugino, R. Ozono, S. Kurisu et al., "Apoptosis is not increased in myocardium overexpressing type 2 angiotensin II receptor in transgenic mice," *Hypertension*, vol. 37, no. 6, pp. 1394–1398, 2001.
- [20] M. Nakayama, X. Yan, R. L. Price et al., "Chronic ventricular myocyte-specific overexpression of angiotensin II type 2 receptor results in intrinsic myocyte contractile dysfunction," *American Journal of Physiology*, vol. 288, no. 1, pp. H317–H327, 2005.
- [21] X. Yan, A. J. T. Schuldt, R. L. Price et al., "Pressure overload-induced hypertrophy in transgenic mice selectively overexpressing AT₂ receptors in ventricular myocytes," *American Journal of Physiology*, vol. 294, no. 3, pp. H1274–H1281, 2008.
- [22] T. Senbonmatsu, S. Ichihara, E. Price, F. A. Gaffney, and T. Inagami, "Evidence for angiotensin II type 2 receptor-mediated cardiac myocyte enlargement during in vivo pressure overload," *Journal of Clinical Investigation*, vol. 106, no. 3, pp. R25–R29, 2000.
- [23] S. Ichihara, T. Senbonmatsu, E. Price, T. Ichiki, F. A. Gaffney, and T. Inagami, "Angiotensin II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II-induced hypertension," *Circulation*, vol. 104, no. 3, pp. 346–351, 2001.
- [24] M. Akishita, M. Iwai, L. Wu et al., "Inhibitory effect of angiotensin II type 2 receptor on coronary arterial remodeling after aortic banding in mice," *Circulation*, vol. 102, no. 14, pp. 1684–1689, 2000.
- [25] Y. Adachi, Y. Saito, I. Kishimoto et al., "Angiotensin II type 2 receptor deficiency exacerbates heart failure and reduces survival after acute myocardial infarction in mice," *Circulation*, vol. 107, no. 19, pp. 2406–2408, 2003.
- [26] Y. Tsutsumi, H. Matsubara, N. Ohkubo et al., "Angiotensin II type 2 receptor is upregulated in human heart with interstitial fibrosis, and cardiac fibroblasts are the major cell type for its expression," *Circulation Research*, vol. 83, no. 10, pp. 1035–1046, 1998.
- [27] M. Stoll, U. M. Steckelings, M. Paul, S. P. Bottari, R. Metzger, and T. Unger, "The angiotensin AT₂-receptor mediates inhibition of cell proliferation in coronary endothelial cells," *Journal of Clinical Investigation*, vol. 95, no. 2, pp. 651–657, 1995.
- [28] C. A. M. Van Kesteren, H. A. A. Van Heugten, J. M. J. Lamers, P. R. Saxena, M. A. D. H. Schalekamp, and A. H. J. Danser, "Angiotensin II-mediated growth and antigrowth effects in cultured neonatal rat cardiac myocytes and fibroblasts," *Journal of Molecular and Cellular Cardiology*, vol. 29, no. 8, pp. 2147–2157, 1997.
- [29] M. Nakajima, H. G. Hutchinson, M. Fujinaga et al., "The angiotensin II type 2 (AT₂) receptor antagonizes the growth effects of the AT₁ receptor: gain-of-function study using gene transfer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 23, pp. 10663–10667, 1995.
- [30] A. D'Amore, M. J. Black, and W. G. Thomas, "The angiotensin II type 2 receptor causes constitutive growth of cardiomyocytes and does not antagonize angiotensin II type 1 receptor-mediated hypertrophy," *Hypertension*, vol. 46, no. 6, pp. 1347–1354, 2005.
- [31] R. E. Widdop, E. S. Jones, R. E. Hannan, and T. A. Gaspari, "Angiotensin AT₂ receptors: cardiovascular hope or hype?" *British Journal of Pharmacology*, vol. 140, no. 5, pp. 809–824, 2003.
- [32] N. Ohkubo, H. Matsubara, Y. Nozawa et al., "Angiotensin type 2 receptors are reexpressed by cardiac fibroblasts from failing myopathic hamster hearts and inhibit cell growth and fibrillar collagen metabolism," *Circulation*, vol. 96, no. 11, pp. 3954–3962, 1997.
- [33] J. Wharton, K. Morgan, R. A. D. Rutherford et al., "Differential distribution of angiotensin AT₂ receptors in the normal and failing human heart," *Journal of Pharmacology and Experimental Therapeutics*, vol. 284, no. 1, pp. 323–336, 1998.