Canopy 2 attenuates the transition from compensatory hypertrophy to dilated heart failure in hypertrophic cardiomyopathy

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Aims
A mismatch between adequate angiogenesis and overgrowth of myocytes may be a critical mechanism controlling the transition from adaptive hypertrophy to heart failure. Canopy 2 (CNPY2) was recently identified as a secreted, HIF-1α-regulated angiogenic growth factor. As angiogenic factors play important roles in the development of myocardial hypertrophy, we investigated the role of CNPY2 in molecular and functional changes during development of chronic heart failure using cardiac-specific transgenic (TG) mice that overexpress human CNPY2.

Methods and results
We generated TG mice that constitutively express CNPY2 in the myocardium. Cardiomyopathy was induced in TG and wild-type (WT) mice by transverse aortic constriction (TAC). WT mice developed significant ventricular hypertrophy at 4 weeks and severe dilatation and heart failure at 12 weeks after TAC. However, TG mice preserved much better cardiac structure and function, with less severe ventricular dilatation and markedly reduced cardiac apoptosis and fibrosis following TAC. Excess CNPY2 in TG mice prevented significant loss of vasculature up to 12 weeks after TAC injury, resulting in a better local myocardial environment that facilitated myocyte survival and prevented excessive matrix remodelling compared with WT mice. TG mice had less accumulation of endogenous tumor suppressor p53 after TAC, indicating intrinsic activation of the p53-mediated repression of HIF-1α, and Cnpy2 was diminished in TG mice compared with WT controls.

Conclusion
Our study showed a correlation between downregulation of endogenous mouse Cnpy2 and p53-mediated HIF-1α inhibition during late-stage hypertrophic development. Additional CNPY2 attenuated the transition from compensatory hypertrophic response to maladaptive ventricular dilatation and heart failure.

Keywords
Angiogenesis • CNPY2 • HIF-1α • p53 • Hypertrophy • Heart failure

Translational perspective
During ischaemic injury, cardiac tissue undergoes compensatory hypertrophy to preserve function, but this hypertrophy can progress to adverse ventricular dilatation and eventually to heart failure. Increased angiogenesis, requiring secreted angiogenic growth factors, may be critical to maintaining beneficial compensatory hypertrophy and preventing adverse cardiac remodelling. We generated mice that overexpress the newly discovered angiogenic factor CNPY2 in heart tissue and showed that they had reduced ventricular dilatation and adverse remodelling after ischaemic injury, with better preserved systolic and diastolic heart function and cardiac vasculature than normal controls. Moreover, we showed that transgenic mice that overexpress CNPY2 had decreased p53 activation in hearts after injury and better maintained expression of the p53 target Hif-1α, thereby potentially retaining a beneficial pro-angiogenic programme. These results suggest that CNPY2 may be a useful component of angiogenic therapy delivered to injured hearts and may help to better preserve their cardiac function and prevent the switch from beneficial compensatory hypertrophy to ventricular dilatation and eventual heart failure.

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Introduction

Various cardiovascular pathologies, such as hypertension and ischaemia, can stress ventricular function by increasing haemodynamic stresses. When this occurs, cardiac performance is initially maintained by beneficial compensatory increase in myocyte size (hypertrophy). However, when haemodynamic overload becomes chronic, myocyte apoptosis, severe interstitial fibrosis, and detrimental matrix remodelling can lead to ventricular dilatation and heart failure. Several cellular pathways have been implicated in this beneficial-to-adverse hypertrophic transition, including accumulation of p53, which can inhibit pathways that are beneficial to the development of cardiac hypertrophy. We found that CNPY2 promoted early-stage beneficial hypertrophy and avoided the transition to heart failure. Pro-angiogenic therapy using factors tailored to support compensatory hypertrophy may be beneficial.

To discover new angiogenic factors for potential therapeutic use, we screened human smooth muscle cells for genes that were differentially regulated during hypoxia and found Canopy 2 (CNPY2), which is induced by HIF-1α under low oxygen and has significant pro-migration and pro-angiogenic function in smooth muscle cells. Previous to our work, little was known about CNPY2 function, but ectopically overexpressed CNPY2 was shown to enhance neurite outgrowth, cell spreading, and migration in vitro and to influence the expression of low-density lipoprotein receptor and FGF21, indicating a role for CNPY2 in cell–cell signalling.

We recently provided a comprehensive tissue distribution profile of mouse Cnpy2 and demonstrated that it is highly expressed in the heart. However, the role of CNPY2 in the myocardium and vasculature is unknown. In this study, we generated transgenic (TG) mice that overexpress human CNPY2 in cardiac tissue to supplement CNPY2 loss after transverse aortic constriction (TAC) to investigate CNPY2’s effect on the chronic development of cardiac hypertrophy. We found that CNPY2 promoted early-stage beneficial hypertrophic responses and prevented later stages of ventricular dilatation and heart failure.

Methods

Experimental details are outlined in the Supplementary material.

Transgenic mice

All animal studies were approved by the Animal Care Committee of the University Health Network and performed in accordance with the ‘Guide for the Care and Use of Laboratory Animals, 8th edition’ (NIH, revised 2011). We generated cardiac-specific TG mice on the C57BL/6 background that carry human CNPY2 driven by the α-myosin heavy chain (α-MHC) promoter. The TG construct also expressed EGFP, and human CNPY2 and endogenous mouse Cnpy2 could be differentiated by species-specific polymerase chain reaction (PCR) primers. Details of the TG construct and its restriction analysis are shown in Supplementary material online, Figure S1 and Tables S1 and S2.

A total of 86 wild-type (WT) C57BL/6 and 88 TG mice were used for this study. To characterize the TG mouse, cardiac expressions of human CNPY2, mouse Cnpy2, and EGFP mRNA were evaluated by reverse transcriptase (RT)–PCR, and CNPY2 and EGFP cardiac protein expression were evaluated by western blot (WT: n = 3, TG: n = 4). CNPY2 concentration in the blood of WT and TG mice was evaluated by enzyme-linked immunosorbent assay (ELISA) (n = 8/genotype), and the TG mice used for these experiments were also used to examine the CNPY2 and EGFP protein expression in various organs by western blot. CNPY2 and EGFP immunostaining was performed in one TG individual.

Experimental design and transverse aortic constriction model

To examine the role of CNPY2 in the development of left ventricular (LV) hypertrophy, LV pressure overload was induced in TG and WT mice using TAC by partially ligating the aorta between the innominate and left common carotid arteries, causing its constant and permanent constriction. Cardiac function was evaluated using two methods: (i) serial echocardiography on the same animals at 0, 1, 2, 4, 8, and 12 weeks after TAC (n = 5/genotype and (ii) terminal pressure–volume (P–V) analysis at 1, 4, and 12 weeks post-TAC in WT and TG animals and compared with sham surgery controls (n = 5/genotype/timepoint for both sham and TAC).

For morphological and immunohistochemical experiments, a different set of WT and TG animals was sacrificed 4 and 12 weeks after TAC (n = 5/genotype/timepoint). Five WT and 5 TG animals sacrificed immediately after sham surgery (0 weeks) were used as controls. The hearts were sectioned, morphology was assessed by haematoxylin and eosin (H&E) staining, angiogenesis was evaluated by immunohistochemical staining against isoelectric and α-smooth muscle actin (α-SMA), fibrosis was evaluated by picrosirius red staining, and apoptosis was evaluated by TUNEL.

To investigate possible mechanisms by which CNPY2 alters ventricular function, p53, Hif-1α, mouse Cnpy2, and human CNPY2 mRNA and protein expression were evaluated at 0, 3, 7, 21, and 56 days after TAC in WT and TG mice by real-time RT–PCR and western blot (n = 5/genotype/timepoint). To evaluate cardiac remodelling, TGFβ1, Col1α, Mmp9, and Mmp2 expressions were evaluated by RT–PCR, and Col1α and Col3α expressions were evaluated by real-time RT–PCR at 0, 3, 7, 21, and 56 days after TAC in WT and TG mice using the same RNA and protein samples. These protein samples were also used to evaluate Mmp9 enzymatic activity by zymography in WT and TG animals 0, 3, 7, and 21 days after TAC. Hif-1α, Vegfa, and Cnpy2 transcript expressions were also evaluated in WT mice at the same timepoints by RT–PCR (n = 3/genotype/timepoint, using a subset of the same mice).

Statistics

Data are presented as mean ± standard deviation (SD). A sample size of five mice/experimental group was determined to provide sufficient power. The echocardiographic measures, which evaluated the same animals at different timepoints, were analysed by linear regression models adjusted for repeated measures using SAS v9.3 (SAS Institute). All other statistical analyses were performed using GraphPad Prism 5 (GraphPad). A two-tailed Student’s t-test was used to compare CNPY2 levels in blood serum in WT and TG mice. One-way analysis of variances (ANOVAs) followed by Tukey’s post hoc tests were used to evaluate differences in gene expression over time in WT mice. All other comparisons were analysed by two-tailed ANOVAs, followed by Bonferroni post hoc tests. Differences were considered statistically significant at P < 0.05.

Results

Endogenous mouse Cnpy2 expression decreases after transverse aortic constriction

Cardiac hypertrophy in WT mice was induced by TAC, and gene expression in myocardial tissues was analysed by RT–PCR at
various timepoints post-surgery. As shown in Figure 1A, compared with control (day 0), Hif-1α was rapidly induced at day 3, peaked at day 7, and gradually decreased from days 21 to 56 ($P < 0.01$ for days 3–56 vs. day 0). Concomitantly, the known Hif-1α-activated angiogenic growth factor Vegfa was also upregulated following TAC ($P < 0.01$ for days 3, 7, and 21 vs. day 0). At the same time, a more dramatic induction of endogenous mouse Cnpy2 was found at days 3 and 7 ($P < 0.01$ vs. day 0), suggesting an active association with the hypertrophic response. As Cnpy2 is transcriptionally controlled by HIF-1α, we hypothesized that it might

Figure 1 Characterization of cardiac-specific TG mice that overexpress Cnpy2 to supplement decreased Cnpy2 expression after TAC. (A) Hif-1α was expressed in the mouse heart before TAC by RT–PCR (day 0) but was upregulated at days 3 and 7, before gradually decreasing at days 21 and 56 after TAC ($n = 3$, one-way ANOVA followed by Tukey’s post hoc tests). Concomitantly, Cnpy2 was dramatically induced starting from day 3, peaking at day 7, and decreased afterwards ($n = 3$, one-way ANOVA followed by Tukey’s post hoc tests). Gapdh served as a loading control and Vegfa as a positive control ($n = 3$, one-way ANOVA followed by Tukey’s post hoc tests). Values are expressed as the average ratio of densitometry of gene/Gapdh. (B) Cnpy2 transgene construct schematic. Human full-length Cnpy2 cDNA, followed by the internal ribosome entry site 2 (IRES2) for the co-expression of EGFP, was cloned into an expression vector under the α-MHC promoter. (C) Genotyping PCR on tail genomic DNA can distinguish TG and WT mice with human-specific Cnpy2 primers, and primers for EGFP. (D) RT–PCR can distinguish endogenous mouse Cnpy2 and exogenous human Cnpy2 RNA expression in the mouse heart with human- and mouse-specific Cnpy2 primers. EGFP was only expressed in TG hearts. (E) Western blotting showed that TG mice had much higher Cnpy2 expression in the heart than WT. F1 mice from four different founders are shown. (F) Cnpy2 expression in different organs of TG mice by western blot. The combination of endogenous mouse Cnpy2 and exogenous human Cnpy2 was detected in the heart, and endogenous Cnpy2 expression is seen in the liver and uterus. EGFP was detected only in the heart, indicating cardiac-specific expression of human Cnpy2. (G) ELISA showed that the levels of secreted Cnpy2 in the blood serum of TG mice were slightly higher than those in WT ($n = 8$, Student’s t-test). (H) Immunostaining showed the basal level of endogenous mouse Cnpy2 (red) in the WT myocardium (top) and the combination of endogenous mouse Cnpy2 and exogenously expressed human Cnpy2 (red) in the myocardium in TG mice (bottom). EGFP was only expressed in TG hearts. Scale bar = 50 μm.
Figure 2: Overexpression of CNPY2 in TG mice prevented late-stage ventricular dilatation after TAC. (A) Morphology of whole hearts (left) and serial sections (right) of TG and WT mice following TAC at 4 and 12 weeks. (B) H&E-stained heart mid-sections. Compared with sham controls, both WT and TG mice developed slightly hypertrophic myocardia in early stages post-TAC (4 weeks). At later stages (12 weeks), WT hearts are severely dilated and enlarged, whereas this effect was almost absent in the TG hearts. (C) WT mice developed larger hearts by 12 weeks after TAC compared with TG hearts (n = 5, two-way ANOVA followed by Bonferroni post hoc tests). (D) Whole heart cross-sectional circumference and area were smaller in TG mice than in WT mice by 12 weeks post-TAC (two-way ANOVA followed by Bonferroni post hoc tests). (F) LV free wall thickness was larger in WT mice than in TG at 4 weeks, but was thinner by 12 weeks, indicating that the initial hypertrophic development of WT cardiac muscles in the early stages transitioned into a more severe LV dilatation at later stages compared with TG hearts (two-way ANOVA followed by Bonferroni post hoc tests). (G) No difference in LV free wall circumference was seen between WT and TG at 4 weeks, but WT showed a larger circumference than TG mice due to increased ventricular dilatation at 12 weeks (two-way ANOVA followed by Bonferroni post hoc tests). (H) LV free wall area was larger in WT than in TG at 4 weeks, but was similar at 12 weeks (two-way ANOVA followed by Bonferroni post hoc tests).
play a role in mediating the transition from early adaptive hypertrophic signalling to the later maladaptive stages that lead to heart failure.

Cardiac-specific CNPY2 TG mice

We generated cardiac-specific TG mice, which carry the human CNPY2 cDNA and EGFP driven by the \(\alpha\)-MHC promoter (Figure 1B and Supplementary material online, Figure S1 and Tables S1–S3). TG mice and their WT littermates can be distinguished by tail genomic DNA genotyping PCR (Figure 1C), as well as by mRNA and protein expression levels (determined by RT–PCR, Figure 1D, by western blot of myocardial tissue, Figure 1E, and in comparison with other organs, Figure 1F). The TG mice showed no noticeable difference in general morphology, body weight, or baseline cardiac function compared with WT at 8–10 weeks (Supplementary material online, Table S4), with only a

Figure 3 Cardiac function was improved in TG mice compared with WT after TAC. At various timepoints following TAC, cardiac systolic function parameters were measured by echocardiography (\(n = 5\), linear regression models adjusted for repeated measures). (A) Fractional shortening [comparison of means: \(P < 0.001\); comparison of the change with time (slope): \(P = 0.001\)], (B) PWT [comparison of means: \(P = 0.002\); comparison of change with time (slope): \(P = 0.008\)], (C) LVIDs [comparison of means: \(P < 0.001\); comparison of change with time (slope): \(P < 0.001\)], and (D) LVIDd [comparison of means: \(P < 0.001\); comparison of change with time (slope): \(P < 0.001\)] were all significantly improved in TG compared with WT mice over 12 weeks post-TAC. \(P\)-V analysis measured diastolic function at 1, 4, and 12 weeks post-TAC (\(n = 5\), two-way ANOVA followed by Bonferroni post hoc tests). (E) \(dp/dt_{\text{min}}\), and (F) Tau were significantly improved in TG mice compared with WT from 4 weeks post-TAC.
Figure 4  TG mice had better preserved vasculature following TAC. (A) Isolectin IB4 (red) staining for capillary density demonstrated that TG mice preserved a more extensive vasculature network than WT mice 12 weeks after TAC (n = 5). Transverse (top) and longitudinal (bottom) images are shown, and blue is 4′,6-diamidino-2-phenylindole (DAPI)-stained nuclei. Values are expressed as the percentage isolecin IB4-positive/total area (left) and as the number of capillary structures/mm² (right, two-way ANOVAs followed by Bonferroni post hoc tests). (B) α-SMA staining (red) for arteriolar density indicated that TG mice had more mature arteriolar structures at both 4 and 12 weeks after TAC than those in WT mice (n = 5). WT and TG sham surgery controls had similar arteriolar density. Values are expressed as the percentage α-SMA-positive area/total area of the LV free wall (top) and as the number of α-SMA-positive lumenal structures/mm² (bottom, two-way ANOVAs followed by Bonferroni post hoc tests). DAPI-stained nuclei are shown in blue and green is autofluorescence. Scale bars = 50 μm.
slightly higher level of secreted CNPY2 detected in the blood by ELISA (*P < 0.05, Figure 1G*). Immunohistochemical staining demonstrated expression of endogenous mouse Cnpy2 in the WT myocardium and much higher CNPY2 expression (the combination of the human exogenous CNPY2 and endogenous mouse Cnpy2) in the myocardium of TG animals. EGFP was only expressed in the TG mice (*Figure 1H*).

**CNPY2 prevented transition from hypertrophic growth to ventricular dilatation**

As shown by whole heart morphology, serial sections (*Figure 2A*), and H&E-stained heart mid-sections (*Figure 2B*), WT mice developed hypertrophy, including a general enlargement of the whole
heart and thickening of the LV wall in early stages (4 weeks) following TAC and further enlarged but thinner and dilated LVs at later stages (12 weeks). In contrast, although the TG mice also developed some early hypertrophy, further cardiac enlargement and ventricular dilatation were nearly absent at later stages. The heart-to-body weight ratio was significantly reduced in TG mice compared with WT at 12 weeks post-surgery (Figure 2C, \( P < 0.01 \)). Whole heart cross-sectional circumference (Figure 2D) and whole heart cross-sectional area (Figure 2E) were also reduced in TG mice compared with WT at 12 weeks (\( P < 0.01 \) for both parameters). The LV free walls of TG mice were significantly thinner than those of WT 4 weeks after TAC but significantly thicker at 12 weeks (Figure 2F, \( P < 0.01 \) at both timepoints). No significant difference could be found in the LV free wall circumference between the WT and TG mice at 4 weeks, but WT mice had increased circumference compared with TG mice at 12 weeks, indicating that the LV dilatation seen in late-stage WT mice was reduced in TG mice (Figure 2G, \( P < 0.01 \)). The total LV free wall area was smaller in TG mice than that in WT at 4 weeks, indicating less compensatory hypertrophy in the TG animals at this stage (\( P < 0.05 \)), but at 12 weeks, there was no significant difference (Figure 2H). Taken together, TG mice developed moderate cardiac hypertrophy at early timepoints (4 weeks) post-TAC, but further ventricular dilatation was almost completely inhibited at later stages (12 weeks), in contrast to the larger dilated hearts with thinner LV free walls observed in WT mice at 12 weeks post-TAC.

**CNPY2 maintained and improved cardiac function**

To assess whether the better preserved morphology in TG mice correlated with functional improvement compared with WT after TAC, cardiac systolic function was evaluated by echocardiography. TG mice had superior fractional shortening compared with WT over 12 weeks post-TAC (Figure 3A, comparison of both means and slope: \( P < 0.01 \)). Posterior wall thickness (PWT) was reduced in TG mice compared with WT (Figure 3B, comparison of both means and slope: \( P < 0.01 \)). LV internal systolic (LVIDs) and diastolic (LVIDd) diameters were smaller in TG mice post-TAC compared with WT (Figure 3C and D, \( P < 0.01 \) for both means and slope of both parameters).

Diastolic heart function was evaluated by \( P–V \) analysis. For both dp/dt\(_{max}\) and Tau, TG mice had significantly preserved heart function compared with WT at 4 and 12 weeks post-TAC (Figure 3E and F, \( P < 0.05 \) for Tau at 4 and 12 weeks and dp/dt\(_{max}\) at 12 weeks, \( P < 0.01 \) for dp/dt\(_{max}\) at 4 weeks). Taken together, although diastolic function was similar in WT and TG mice during the initial hypertrophic response (1–2 weeks), it became gradually jeopardized following TAC in WT mice when transiting from the early adaptive phase to later stages of ventricular dilatation and heart failure. In contrast, TG mice exhibited only slight ventricular dilatation at later timepoints, with enhanced cardiac function compared with WT throughout the later stages of hypertrophic remodelling.

**CNPY2 maintained vasculature density and structure**

As angiogenesis is an important contributor to the transition from beneficial hypertrophy to later stages of maladaptive ventricular dilatation, \(^7\) capillary structure density was assessed using isoelectric IB4 staining, which revealed a dramatic decrease in the density and number of capillary structures at later stages (12 weeks) post-TAC compared with early stages (4 weeks) in WT mice. However, TG mice maintained similar capillary density and number at 12 weeks compared with 4 weeks (\( P < 0.05 \) TG vs. WT for both parameters, Figure 4A). \( \alpha \)-SMA staining was used to identify arteriolar structures at 4 and 12 weeks post-TAC. Compared with WT, TG hearts had greater \( \alpha \)-SMA expression by area (\( P < 0.01 \)) and by number of stained lumenal structures (\( P < 0.05 \)) at 4 and 12 weeks post-TAC, despite no difference between WT and TG sham surgery controls, indicating no differences in cardiac vasculature in the absence of banding (Figure 4B). These data suggest that increased CNPY2 in TG mice maintained the relative abundance of \( \alpha \)-SMA-positive arteriolar and isocitrate-positive capillary structures through neovascularization or enhanced preservation of existing vessels.

We hypothesized that the better preserved vasculature seen in TG mice compared with WT post-TAC may result in reduced cardiac apoptosis, thereby maintaining heart function. Indeed, we saw reduced numbers of TUNEL-positive cells at 4 (\( P < 0.01 \)) and 12 weeks (\( P < 0.05 \), Supplementary material online, Figure S2A) and reduced caspase-3- and -9 activity at 4–8 weeks (\( P < 0.01 \)) and 12 weeks (\( P < 0.01 \) for caspase-3 and \( P < 0.05 \) for caspase-9, Supplementary material online, Figure S2B) post-TAC in TG mice compared with WT.
Increased CNPY2-induced angiogenesis may also reduce adverse fibrosis after TAC. RT–qPCR demonstrated that markers of fibrosis and matrix disruption are increased in WT mice post-TAC (P < 0.01 for days 3–56 for Tgf b1 and Col1, days 3 and 21 for Mmp2, and days 3–21 for Mmp9 vs. day 0 and P < 0.05 for Mmp2 at day 7 vs. day 0, Supplementary material online, Figure S3A). TG mice show upregulation of Col1a and Col3a by RT–qPCR post-TAC, but this induction was reduced compared with WT (Col1a: P < 0.01 for days 3–21, P < 0.05 for day 56 and Col3a: P < 0.01 for days 3–21), and the Col1a/3a mRNA expression ratio is significantly reduced in TG mice (P < 0.01 for all timepoints post-TAC, Supplementary material online, Figure S3B). Picrosirius red staining confirmed decreased total collagen deposition (P < 0.05) and a decreased proportion of thick-to-total fibres in TG mice at 12 weeks compared with WT mice (P < 0.01, Supplementary material online, Figure S3C). Matrix disruption was also decreased in TG mice, as evidenced by decreased Mmp9 activity assayed by zymography (P < 0.01 vs. WT at days 3 and 7 post-TAC, Supplementary material online, Figure S3D). These results suggest that TG mice have reduced adverse cardiac remodelling after TAC compared with WT mice.

**CNPY2 prevents the p53-mediated inhibition of HIF-1α indicative of late-stage heart dilatation**

As shown by real-time RT–PCR (Figure 5A), p53 transcript expression was significantly decreased in TG animals from 7 days post-TAC compared with WT (P < 0.01). Correspondingly, Hif-1α expression
was increased at days 21 and 56 in TG mice compared with WT (P < 0.01, Figure 5B). Endogenous mouse Cnpy2 was dramatically induced post-TAC in TG mice, resulting in approximately two- to four-fold more Cnpy2 in TG mice than that in WT at days 21–56 (P < 0.01, Figure 5C). Exogenous human CNPY2 was uniquely expressed in TG mice under the control of the α-MHC promoter and was not affected by TAC (Figure 5D). Western blotting showed that p35 protein was largely absent in sham controls at day 0, but accumulated in WT animals starting at day 7, peaking at day 21, and remained elevated at day 56 (Figure 5E). In contrast, p35 was consistently inhibited in TG animals (P < 0.01 for WT vs. TG from days 7 to 56). Hif-1α protein was induced by TAC at day 3, peaking at day 7, and decreased afterwards in WT mice, whereas it was consistently upregulated from day 3 in TG, resulting in significantly increased expression at days 21 and 56 after TAC in TG mice compared with WT (P < 0.01). Unlike real-time PCR in which human- or mouse-specific primers and probes were used, our CNPY2 antibody cross-reacts with endogenous mouse Cnpy2 and exogenous human CNPY2, resulting in more total CNPY2 expression in TG mice at all stages following TAC (P < 0.01).

Discussion

TAC-induced hypertrophy can be divided into two distinct stages: the early beneficial hypertrophic response and the later, detrimental stage of ventricular dilation and eventual heart failure. Multiple signalling pathways impact on this progression, and one crucial mechanism mediating the transition from beneficial to deleterious is accumulation of p35 due to chronic haemodynamic pressure and stress on the ventricular wall. Elevated p35 inhibits HIF-1α, resulting in diminished expression of various HIF-1α-controlled angiogenic growth factors. We recently demonstrated that CNPY2 is a novel secreted angiogenic factor that promotes smooth muscle cell migration and is transcriptionally regulated by HIF-1α. Therefore, CNPY2 expression in cardiac tissue would be predicted to decrease during hypertrophy, which we demonstrated in WT mice post-TAC. The data presented here, along with our previous work on CNPY2’s angiogenic function, also suggest that overexpression of CNPY2 may prevent transition from hypertrophy to dilated cardiomyopathy.

We generated cardiac-specific TG mice that constitutively express CNPY2 under the control of the α-MHC promoter. These mice exhibit consistent expression of CNPY2 that is not affected by TAC. The TG mice developed cardiac hypertrophy similar to WT at early stages post-TAC, but severe ventricular dilation was mitigated, and cardiac systolic and diastolic dysfunction was attenuated during later stages post-TAC. These data suggested that overexpression of CNPY2 can prevent progressive ventricular dilation during increased cardiac afterload.

One possible mechanism by which CNPY2 expression could benefit cardiac function is by increased vascular density. Endogenously expressed mouse Cnpy2 decreased significantly during later stages post-TAC, which might have resulted in insufficient angiogenesis to compensate for cardiomyocyte overgrowth. However, in the TG mice, constitutively expressed human CNPY2 played an important role in supplementing this significant loss during later stages. There were no differences in cardiac arterial density in WT and TG mice in the absence of injury, as has been previously reported for cardiac-specific overexpression of the angiogenic factor angiopoietin-1. However, angiogenesis was activated after TAC, and a much better vascularized myocardial environment was maintained in TG mice from early to later stages after hypertrophy. This vasculature maintenance may have contributed to the reduced expression of the p35 stress response factor, thereby decreasing cell death and consequently fibrosis compared with WT mice (Figure 6).

Taken together, we have provided the first proof-of-concept evidence that CNPY2 attenuates the transition from the early beneficial hypertrophic response to the detrimental late stage of ventricular dilation and heart failure, suggesting that CNPY2 may have therapeutic potential for the treatment of cardiac hypertrophy and perhaps other cardiomyopathies.

Supplementary material

Supplementary material is available at European Heart Journal online.

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