

# Neuregulin1 Improved Cardiac Function in Doxorubicin-Treated Mice with Cardiomyocyte-Specific over expression of a Dominant-Negative PI3Kp110 $\alpha$

Yun Bian<sup>1</sup>, Marcy Silver<sup>1</sup>, Jillian Onufrak<sup>1</sup>, Kalon K.L. Ho<sup>2</sup>, Mark A. Marchionni<sup>3</sup>, Peter M Kang<sup>2</sup>, David A Goukassian<sup>1</sup>, Joseph Carrozza<sup>1</sup>, James P Morgan<sup>1</sup> and Xinhua Yan<sup>1\*</sup>

<sup>1</sup>Cardiovascular Research, Department of Medicine, Steward St. Elizabeth's Medical Center and Tufts University School of Medicine, Boston, USA

<sup>2</sup>Cardiovascular Division, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA

<sup>3</sup>Alzcor Pharmaceuticals Inc. Arlington, USA

## Abstract

Neuregulin1s (NRG1s) are effective for protecting the heart from various stresses. Our previous studies show that heterozygous knockout of the NRG1 gene worsens while injections of a recombinant NRG1 improve cardiac function in Chemotherapy Drug Doxorubicin (DOX) - treated mice. In cultured cardiomyocytes, studies show that the Phosphoinositide 3-Kinase (PI3K) pathway is necessary for NRG1 to reduce DOX-induced apoptosis and loss of cardiac troponins. Here, we test whether PI3Kp110 $\alpha$ , a Class IA PI3K isoform, is necessary for NRG1's cardioprotective effects in DOX-treated mice. DOX was administered to mice with cardiomyocyte-specific overexpression of a constitutively active PI3Kp110 $\alpha$  (CaPI3K), or a dominant negative PI3Kp110 $\alpha$  (dnPI3K). Solvent or NRG1 was administered concurrently with DOX to dnPI3K mice. Our results showed that survival and cardiac function were improved in CaPI3K, but worsened in dnPI3K mice. Concurrent NRG1 injections significantly improved survival and cardiac function in dnPI3K mice, which were associated with increased activation of ERK1/2 and mTORC1 in the heart. These results have demonstrated that PI3Kp110 $\alpha$  is important for protecting the heart from DOX. Further, the results suggest that PI3Kp110 $\alpha$  is not necessary for NRG1 to protect the heart from DOX. NRG1 may protect the heart by activating alternative survival pathways, such as ERK1/2 and mTORC1, in DOX-treated dnPI3K mouse hearts.

**Keywords:** Neuregulin1; PI3K; Doxorubicin; Cardiotoxicity

## Introduction

The Neuregulin1 (NRG1) gene is a member of the epidermal growth factor gene family [1]. It encodes at least fifteen isoforms of NRG1 proteins, which are ligands of the HER receptors [2]. The NRG1-HER signaling is pivotal for protecting the heart from various cardiac stresses [3-5]. Our results show that heterozygous knockout of the NRG1 gene worsens, whereas injections of a recombinant NRG1 alleviate, DOX-induced cardiac dysfunction in mice [3,6]. In the heart, NRG1 is synthesized and secreted by the endocardium and the endothelium of the cardiac microvasculature [7]. NRG1 activates HER2 and HER4 receptors on cardiomyocytes, thereby activating multiple signaling pathways and regulating key functions of cardiomyocytes [2,7]. A major signaling pathway activated by NRG1-HER is the phosphoinositide 3-kinase (PI3K) pathway [2]. Studies in isolated cardiomyocytes show that PI3Ks are necessary for multiple cardioprotective effects of NRG1, such as anti-apoptosis, antioxidative stress, maintaining cardiac troponins and cardiac repair [6,8]. In addition to PI3Ks, NRG1 also activates multiple signaling pathways, such as ERK, mTOR, PKC and STAT, to exert its functions [2,9].

PI3 kinases are conserved lipid kinases that phosphorylate the 3'-hydroxyl group of phosphoinositides [10]. Class I PI3Ks are most studied in both cancer and the heart. They are further divided into class IA and class IB. Class IA are heterodimers comprised of a regulatory subunit (p85 $\alpha$ , p55 $\alpha$ , p50 $\alpha$ , p85 $\beta$ , p55 $\gamma$ ) and a catalytic subunit (p110 $\alpha$ , p110 $\beta$ , p110 $\delta$ ). In response to the Receptor Tyrosine Kinase (RTKs) activation, class IA PI3Ks are recruited to the tyrosine phosphate motifs on the activated RTKs via the regulatory subunits. Class IB PI3Ks are composed of a regulatory subunit p101 and a catalytic subunit p110 $\gamma$ , and activated by Gprotein Coupled Receptors (GPCRs) [11,12]. PI3Kp110 $\alpha$  is important for protecting the heart

from cardiac stress [13,14]. Studies have shown that cardiomyocyte-specific overexpression of a dominant negative PI3Kp110 $\alpha$  (dnPI3K) exacerbates pressure-overload induced hypertrophic cardiomyopathy and myocardial infarction induced heart failure [13,15]. With ongoing clinical trials using PI3K inhibitors for cancer therapy it is important to know whether inhibition of PI3Kp110 $\alpha$  will aggravate chemotherapy, such as Doxorubicin (DOX), induced cardiotoxicity; and if so, whether there are means to alleviate this type of cardiotoxicity [16].

We hypothesize that inhibition of PI3Kp110 $\alpha$  may exacerbate DOX cardiac dysfunction. NRG1, by activating alternative signaling pathways, may be capable of circumventing the loss of PI3Kp110 $\alpha$  to provide cardioprotective effects against DOX.

To study the specific effects of PI3Kp110 $\alpha$  inhibition in DOX-injured hearts, we tested our hypotheses by using transgenic mouse models with cardiomyocyte-specific overexpression of a constitutively active PI3Kp110 $\alpha$  (CaPI3K) or a dominant negative PI3Kp110 $\alpha$  (dnPI3K). First, we tested whether cardiomyocyte-specific overexpression CaPI3K would improve cardiac function in DOX-treated mice; second, whether cardiomyocyte-specific overexpression

**\*Corresponding author:** Xinhua Yan, Cardiovascular Research, St. Elizabeth's Medical Center, 736 Cambridge St. CBR345, Boston, MA 02135, Tel: 001-617-562-7608; E-mail: [xinhua.yan@tufts.edu](mailto:xinhua.yan@tufts.edu)

**Received** July 30, 2013; **Accepted** August 22, 2013; **Published** August 29, 2013

**Citation:** Bian Y, Silver M, Onufrak J, Ho KKL, Marchionni MA, et al. (2013) Neuregulin1 Improved Cardiac Function in Doxorubicin-Treated Mice with Cardiomyocyte-Specific over expression of a Dominant-Negative PI3Kp110 $\alpha$ . J Cardiovasc Dis Diagn 1: 120. doi:10.4172/2329-9517.1000120

**Copyright:** © 2013 Bian Y, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

of dnPI3K would exacerbate cardiac dysfunction in DOX-treated mice; and third, whether NRG1 injections would alleviate DOX cardiac dysfunction in dnPI3K mice.

## Methods

### Animal model

The generation of CaPI3K and dnPI3K mice were described previously [17]. Three month old WT and CaPI3K male mice (in total, n=106) were treated with a single dose of DOX (15 mg/kg, i.p. Bedford Laboratories, Bedford, OH). Three-month old WT and dnPI3K female mice (in total, n=201) were treated with a single dose of DOX (20 mg/kg, i.p.), or a concurrent treatment of NRG1 and DOX. Solvent (20 mM sodium acetate, 100 mM sodium sulfate, 1% mannitol and 100 mM L-arginine, pH 6.5) or recombinant NRG1 $\beta$  (recombinant human glial growth factor 2 (rhGGF2), 0.75 mg/kg s.c.; a gift from Acorda Therapeutics Inc.) was administered one day before and daily after the DOX administration. All animal studies were in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Institute of Health Publication, 1996) and approved by Steward St. Elizabeth's Medical Center IACUC.

### Left ventricular function

Function was measured by LV catheterization using Millar Mikro-Tip Blood Pressure System (AD Instruments, Inc., Colorado Springs, CO). In brief, a Millar Mikro-Tip catheter transducer was inserted into the LV via the right carotid artery after mice were anesthetized with urethane (1000mg/kg) and  $\alpha$ -chloralose (50mg/kg). LV systolic and diastolic pressures were recorded. The time point chosen was based on pilot experiments in each mouse line.

### Western blot analysis

Mice LV tissue (n=4-6 per group) was lysed in a buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10 mM sodium pyrophosphate, 20mM  $\beta$ -glycerophosphate, 10 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM NaF, 1 mM PMSF, and protease inhibitor cocktail tablet (Roche Diagnostics Corporation, Indianapolis, IN). LV tissue was homogenized by PowerGene homogenizer (Fisher Scientific Pittsburgh, PA). Proteins were quantified using the Bradford Assay (Bio-Rad Laboratories, Hercules, CA). LV proteins (50  $\mu$ g) were separated by SDS page and transferred to Whatman nitrocellulose membrane (Fisher Scientific, Pittsburgh, PA). Membranes were probed with antibodies against phosphorylated and total Akt (Thr 308 or Ser 473), ERK1/2 (Thr202/Tyr204) and S6 (Ser240/244) (Cell Signaling Technology, Danvers, MA).

### Statistical analysis

Results are presented as Mean  $\pm$  SEM. Statistical analysis was conducted using SigmaStat (Systat Software Inc. San Jose, CA). Comparison between groups was performed by ANOVA followed by Tukey's test. Kaplan-Meier estimates of survival were computed using SAS for Windows v6.12 (SAS Institute, Cary, NC). Differences were considered significant with  $P < 0.05$ .

## Results

### Cardiomyocyte-specific overexpression of CaPI3K improved survival and cardiac function in DOX-treated mice

DOX significantly reduced two-week survival to 14% in WT-DOX (5 out of 37 mice survived,  $P < 0.05$ ) compared to non-treated WT

and CaPI3K mice (20 out of 20 mice survived per group). CaPI3K overexpression significantly improved survival to 38% in CaPI3K-DOX (6 out of 16 mice survived,  $P < 0.05$ ) compared to WT-DOX mice (Figure 1A).

In non-DOX treated mice, heart weight normalized by tibial length (HW/TL) was significantly higher in CaPI3K vs. WT mice; cardiac function was similar between CaPI3K and WT mice (Figures 1B-1F, Table 1). DOX reduced body weight (BW) and HW/TL in both WT-DOX and CaPI3K-DOX mice. HW/TL, however, was significantly higher in CaPI3K-DOX vs. WT-DOX mice (Figures 1B and 1C, Table 1). Five days after the injection, DOX significantly reduced LV systolic pressure (LVSP), dp/dtmax and dp/dtmin in WT-DOX mice, while these indices were maintained in CaPI3K-DOX mice and were significantly higher in CaPI3K-DOX compared to WT-DOX mice (Figures 1D-1F and Table 1).

We measured the activation of PI3Ks (readout: pAkt-Thr308), ERK1/2, mTORC1 (readout: pS6) and mTORC2 (readout: pAkt-Ser473) in mouse hearts [18-21]. Without DOX, the PI3K activity was increased 6.5-fold in CaPI3K hearts [17]. pAkt (Thr 308 and Ser473) and pS6 were significantly higher in CaPI3K vs. WT; pERK1/2 were similar in CaPI3K and WT.

Five days after the DOX treatment, pAkt (Thr 308 and Ser473) and pERK1/2 were higher in CaPI3K-DOX vs. WT-DOX (Figures 1G and 1H).

Taken together, these results demonstrated that cardiomyocyte-specific overexpression of CaPI3K improved survival and cardiac function in DOX-treated mice. These were associated with increased activation of PI3K, ERK1/2 and mTORC2 in CaPI3K-DOX vs. WT-DOX mouse hearts.

### Cardiomyocyte-specific overexpression of dnPI3K exacerbated cardiac dysfunction in DOX-treated mice

DOX significantly reduced two-week survival to 21 % in WT-DOX (6 out of 28 mice survived,  $P < 0.05$ ) and 16% in dnPI3K-DOX mice (3 out of 19 mice survived,  $P < 0.05$ ), compared to non-treated WT and dnPI3K mice respectively (20 out of 20 mice survived per group; Figure 2A).

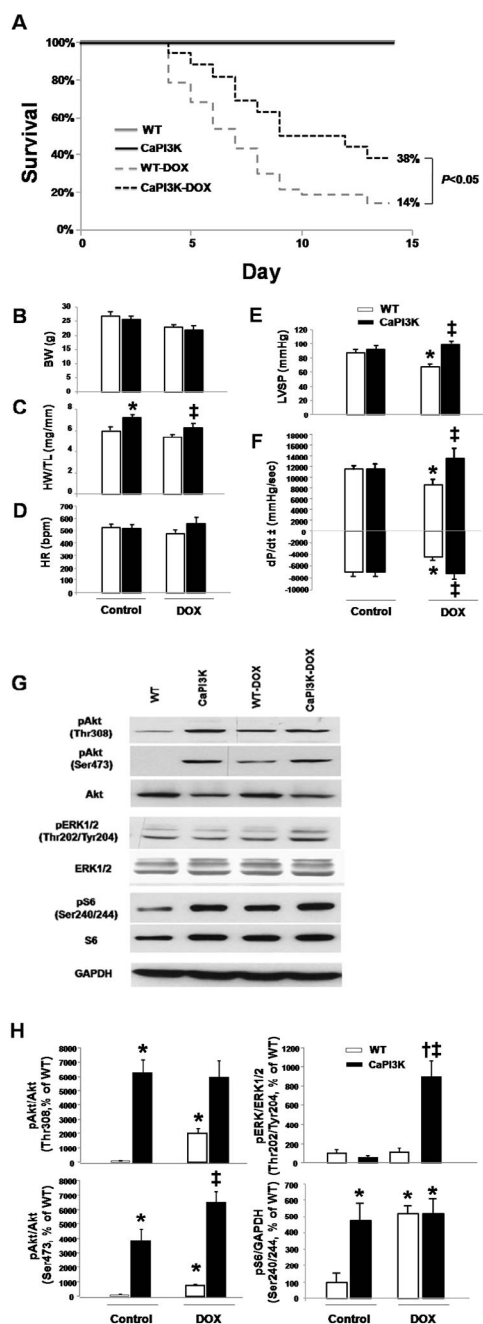
In non-DOX treated mice, HW/TL was lower in dnPI3K vs. WT mice; cardiac function was similar between dnPI3K and WT mice (Figures 2B-2F, Table 2). DOX reduced BW and HW/TL in mice. These indices were significantly lower in dnPI3K-DOX vs. WT-DOX mice (Figures 2B and 2C, Table 2). Six days after the DOX treatment, DOX significantly reduced heart rate (HR), LVSP, dp/dtmax and dp/dtmin in dnPI3K-DOX but not in WT-DOX mice (Figures 2D-2F, Table 2).

Without DOX treatment, PI3K activity was decreased 77% in dnPI3K hearts [17]. pAkt (Thr308 and Ser473) were lower, while pERK1/2 and pS6 were similar, in dnPI3K vs. WT hearts. DOX significantly reduced pERK1/2 and pS6 in dnPI3K-DOX hearts compared to dnPI3K and WT-DOX hearts (Figures 2G and 2H).

These results demonstrated that cardiomyocyte-specific overexpression of dnPI3K exacerbated DOX cardiac dysfunction in mice. This was associated with decreased activation of ERK1/2 and mTORC1 in dnPI3K-DOX compared to WT-DOX hearts.

### NRG1 improved survival and cardiac function in DOX-treated dnPI3K mice

NRG1 injections significantly improved two-week survival to 48%



**Figure 1:** Cardiomyocyte-Specific Overexpression of CaPI3K Improved Survival and Cardiac Function in DOX-Treated Mice (A) Survival analysis. Two-week survival was significantly reduced to 14% in WT-DOX mice (n=37, 5 out of 37 mice survived,  $P<0.05$ ) compared to non-treated WT or CaPI3K mice (n=20 per group). CaPI3K overexpression in cardiomyocytes significantly improved survival to 38% in CaPI3K-DOX mice (n=16, 6 out of 16 mice survived,  $P<0.05$ ) compared to WT-DOX mice. (B) - (C) Body weight (BW) and heart weight to tibial length ratio (HW/TL) in non-treated and DOX-treated mice. (D) - (F) Hemodynamic measurements. Function was measured by LV catheterization five days after the DOX treatment. HR=heart rate; LVSP=left ventricular systolic pressure. BW, HW/TL and function were measured in WT (n=8), CaPI3K (n=8), WT-DOX (n=9) and CaPI3K-DOX (n=8) mice. (G) - (H) Western blot analysis. Phosphorylated and total Akt, ERK1/2 and S6 were measured in WT (n=3), CaPI3K (n=3), WT-DOX (n=4) and CaPI3K-DOX (n=4) hearts five days after the DOX injection. The representative blots (G) and quantitative analysis (H) were shown. \* $P<0.05$  vs. WT; † $P<0.05$  vs. CaPI3K; ‡ $P<0.05$  vs. WT-DOX.

in NRG1-dnPI3K-DOX (15 out of 31 mice survived,  $P<0.05$ ) compared to 16% in dnPI3K-DOX mice. NRG1 also improved survival to 44% in NRG1-WT-DOX (11 out of 25 mice survived,  $P=0.25$ ) compared in 21% in WT-DOX mice. NRG1 alone did not alter survival rate in non-DOX treated mice [6].

NRG1 injections did not alter the adverse effects of DOX on BW and HW/TL in dnPI3KDOX mice (Figures 2B and 2C, Table 2). NRG1, however, significantly improved HR, LVSP, dP/dtmax and dP/dtmin in NRG1-dnPI3K-DOX vs. dnPI3K-DOX mice (Figures 2D-2F and Table 2).

NRG1 injections significantly increased pERK1/2 and pS6 in NRG1-dnPI3K-DOX vs. dnPI3K-DOX hearts (Figure 2G and 2H).

These results demonstrated that NRG1 was capable of improving cardiac function in DOX-treated mice with cardiac-specific inhibition of PI3Kp110 $\alpha$ . This effect of NRG1 was associated with increased activation of ERK1/2 and mTORC1.

## Discussion

This study demonstrated that inhibition of PI3Kp110 $\alpha$  exacerbated DOX-induced cardiac dysfunction in mice; NRG1 injections alleviated DOX cardiac dysfunction in mice with decreased PI3Kp110 $\alpha$  activity in the heart. These results suggest that cautions must be taken when PI3K inhibitors are used in combination with DOX in cancer patients. The results also suggest that NRG1 could be used as an adjuvant agent to reduce the incidence and severity of heart failure caused by anti-cancer therapies that use PI3K inhibitors and DOX.

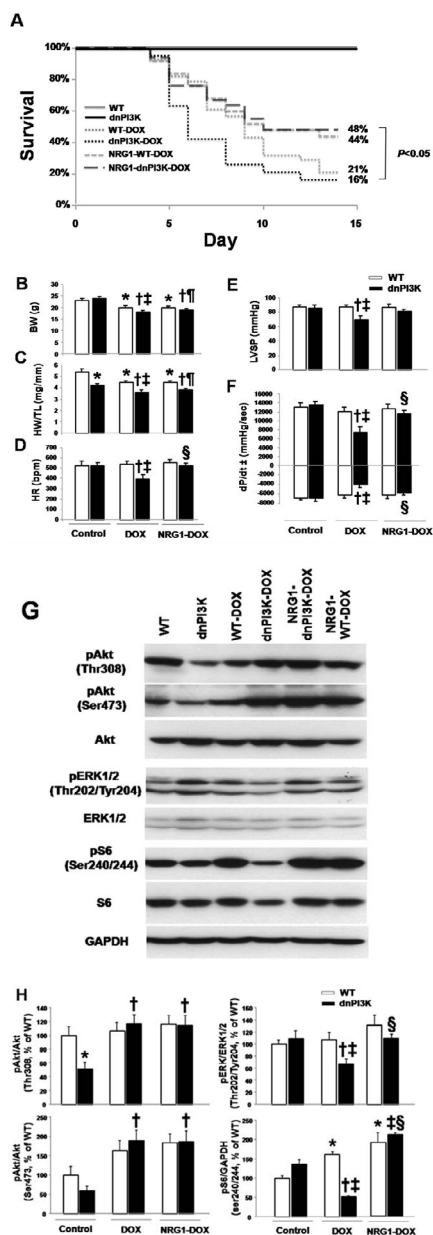
Since its discovery more than 40 years ago, DOX continues to be used as a first-line anti-neoplastic drug for a wide variety of cancers, despite that it causes a dose-related cardiotoxicity [22]. Chemotherapy, such as DOX, is often inevitable in certain cancer patients previously treated with kinase inhibitors. Combination cancer chemotherapy using kinase inhibitors and DOX, however, can cause a high incidence of a severe of heart failure [23,24].

Clinical studies have shown that the incidence of New York Heart Association class III/IV heart failure is 16% among patients treated with Trastuzumab (a monoclonal antibody that blocks HER2) and

	WT (n=8)	CaPI3K (n=8)	WT-DOX (n=9)	CaPI3K-DOX (n=8)
BW (g)	26 ± 2.0	25 ± 1.0	23 ± 1	22 ± 1
TL (mm)	16.3 ± 0.3	16.0 ± 0.2	15.8 ± 0.3	15.6 ± 0.3
HW (mg)	94 ± 6	113 ± 4*	85 ± 4	100 ± 8
LVW (mg)	82 ± 5	98 ± 3*	73 ± 2	87 ± 6
HW/TL (mg/mm)	5.8 ± 0.3	7.1 ± 0.2*	5.4 ± 0.2	6.4 ± 0.4 <sup>†</sup>
LVW/TL (mg/mm)	5.0 ± 0.3	6.2 ± 0.2*	4.7 ± 0.2	5.5 ± 0.4 <sup>†</sup>
HR (beat/mm)	531 ± 22	522 ± 32	479 ± 22	558 ± 50
LVSP (mmHg)	88 ± 5	92 ± 5	68 ± 4*	99 ± 4 <sup>†</sup>
LVEDP (mmHg)	1.5 ± 0.6	1.6 ± 0.5	0.9 ± 0.1	0.6 ± 0.4
dP/dt max (mmHg/sec)	11568 ± 619	11572 ± 956	8564 ± 1019*	13589 ± 1863 <sup>†</sup>
dP/dt min (mmHg/sec)	6826 ± 727	6900 ± 649	4455 ± 519*	7219 ± 926 <sup>†</sup>

BW, body weight; TL, tibial length; HW, heart weight; LVW, left ventricular weight; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; Mean ± SEM; \* $P<0.05$  vs. WT; † $P<0.05$  vs. CaPI3K; ‡ $P<0.05$  vs. WT-DOX.

**Table 1:** Cardiac hemodynamic measurements of DOX-treated CaPI3K mice.



**Figure 2:** Cardiomyocyte-Specific Overexpression of dnPI3K Exacerbated Cardiac Dysfunction in DOX-Treated Mice; Concurrent NRG1 Injections Improved Survival and Cardiac Function in DOX-Treated dnPI3K Mice (A) Survival analysis. DOX reduced two-week survival to 21% in WT-DOX (n=28, 6 out of 28 mice survived) and 16% in dnPI3K-DOX (n=19, 3 out of 19 mice survived). NRG1 injections improved survival to 44% in NRG1-WT-DOX mice (n=25, 11 out of 25 mice survived) and 48% in NRG1-dnPI3K-DOX mice (n=31, 15 out of 31 mice survived). 15 (B) - (C) Body weight (BW) and heart weight to tibial length ratio (HW/TL) in solvent-treated, DOX-treated and NRG1-treated mice six days after the DOX injection. (D) - (F) Hemodynamic measurements. Function was measured by LV catheterization six days after the DOX treatment. HR=heart rate; LVSP=left ventricular systolic pressure. BW, HW/TL and function were measured in WT (n=8), dnPI3K (n=8), WT-DOX (n=13), dnPI3K-DOX (n=12), NRG1-WT-DOX (n=8) and NRG1-dnPI3K-DOX (n=10) mice. (G) - (H) Western blot analysis. Phosphorylated and total Akt, ERK1/2 and S6 were measured in WT (n=5), dnPI3K (n=5), WT-DOX (n=6), dnPI3K-DOX (n=6), NRG1-WT-DOX (n=6) and NRG1-dnPI3K-DOX (n=6) mouse hearts six days after the DOX injection. The representative blots (G) and quantitative analysis (H) were shown. \* $P < 0.05$  vs. WT; † $P < 0.05$  vs. dnPI3K; ‡ $P < 0.05$  vs. WT-DOX; § $P < 0.05$  vs. dnPI3K-DOX; ¶ $P < 0.05$  vs. NRG1-WT-DOX.

concurrent DOX therapy, compared to 3% in patients treated with DOX alone (fivefold increase) [5]. Compared to DOX mono-therapy induced heart failure, which may appear years after the cessation of the therapy [25], cardiac dysfunction caused by Trastuzumab and DOX combination therapy is frequently detected during the course of the treatment and may be irreversible [5,26].

Like the HER2 receptor, the PI3K pathway is one of the most mutated pathways in cancer [27]. PIK3CA, the gene encoding the PI3Kp110 $\alpha$ , is found mutated in 27% of breast, 24% of endometrial and 15% of colorectal cancers, therefore, is a major drug target for cancer therapy [27]. PI3Ks, on the other hand, are pivotal for maintaining cardiac physiological function, especially in the presence of cardiac stress. It is important to determine whether inhibition of PI3Ks could cause cardiac side-effects, especially in the presence of DOX. We used a transgenic mouse model with cardiomyocyte-specific overexpression of a dominant negative PI3Kp110 $\alpha$ , and treated them with DOX. This could mimic the clinical setting where DOX is used followed by PI3Kp110 $\alpha$  inhibitors. We have demonstrated that although inhibition of PI3Kp110 $\alpha$  itself does not cause cardiac dysfunction; these hearts are more susceptible to DOX cardiac toxicity. In addition, we have demonstrated that PI3Kp110 $\alpha$  is not necessary for NRG1 to improve cardiac function in DOX-injured mice. Our results suggest that NRG1 could improve cardiac function in patients treated with DOX and PI3Kp110 $\alpha$ -specific inhibitors.

This study has focused on the complex signaling pathways downstream of NRG1-HER in the heart. We have shown that alternative pathways exist downstream of NRG1 to compensate for the loss of PI3Kp110 $\alpha$ . The activation of ERK1/2 and mTORC1 are decreased in dnPI3K-DOX mouse hearts, but are maintained in NRG1-dnPI3K-DOX hearts, suggesting that NRG1 may circumvent the loss of PI3Kp110 $\alpha$  by activating these ERK1/2 and mTORC1.

mTORC1 is pivotal for protein synthesis and cell growth [28]. Activation of mTORC1, however, inhibits Akt [29]; on the other hand, inhibition of mTORC1 activates Akt and ERK1/2 [18,19]. We have observed that increased activation of mTORC1 and mTORC2 is associated with either an exacerbation or an improvement of cardiac function in DOX-treated mice. Further studies are needed to clarify the role of mTOR in DOX-treated hearts. Although in non-DOX treated mice, the PI3K activity is lower in dnPI3K vs. WT mouse hearts [17], it is increased in dnPI3K-DOX vs. dnPI3K hearts (Figure 2G and 2H). This could be caused by the feedback activation of Akt and mTORC2 caused by decreased activation of mTORC1 in dnPI3K-DOX hearts [20,30].

The role of NRG1 on tumor growth and progression is controversial. Studies have shown that NRG1 is either an oncogene or a tumor-suppressor gene [31,32]. Accumulating evidence has pointed out that the outcome of NRG1 stimulation in tumor cells depends on the integration of the biological effects of NRG1 on tumor cells, as well as the cell type, the expression and density of the HER receptors and their ligands, and the dosage and the frequency of NRG1 stimulation [33-35]. Studies have also shown that NRG1 increases breast cancer sensitivity to DOX. The mechanism may be related to NRG1's effect on inducing the activity of topoisomerase II, which is a major target of DOX [36,37].

Taken together, we have demonstrated that hearts with decreased activation of PI3Kp110 $\alpha$  are more susceptible to DOX-induced cardiac dysfunction. NRG1 is capable of circumventing the partial loss of PI3Kp110 $\alpha$  to improve cardiac function in DOX-injured hearts by

	Control		DOX		NRG1-DOX	
	WT (n=8)	dnPI3K (n=8)	WT (n=13)	dnPI3K (n=12)	WT (n=8)	dnPI3K (n=10)
BW (g)	23 ± 1.0	24 ± 0.4	20 ± 0.3*	18 ± 0.4 <sup>†‡</sup>	20 ± 0.3*	19 ± 0.5 <sup>†¶</sup>
TL (mm)	15.9 ± 0.3	16 ± 0.1	15.5 ± 0.2	15.1 ± 0.3	15.8 ± 0.2	15.3 ± 0.2
HW (mg)	86 ± 5	68 ± 3*	70 ± 2*	54 ± 3 <sup>†‡</sup>	71 ± 2*	59 ± 2 <sup>†¶</sup>
LVW (mg)	69 ± 4	59 ± 3	61 ± 2	45 ± 2 <sup>†‡</sup>	62 ± 2	50 ± 2 <sup>†¶</sup>
HW/TL (mg/mm)	5.4 ± 0.3	4.2 ± 0.2*	4.5 ± 0.1*	3.6 ± 0.2 <sup>†‡</sup>	4.5 ± 0.1*	3.8 ± 0.1 <sup>†¶</sup>
LVW/TL (mg/mm)	4.4 ± 0.2	3.7 ± 0.1	3.9 ± 0.1	3.0 ± 0.1 <sup>†‡</sup>	3.9 ± 0.1	3.3 ± 0.1 <sup>†¶</sup>
HR (beat/mm)	529 ± 43	525 ± 30	541 ± 32	398 ± 40 <sup>†‡</sup>	557 ± 30	529 ± 23 <sup>§</sup>
LVSP (mmHg)	88 ± 3	86 ± 5	88 ± 3	70 ± 6 <sup>†‡</sup>	87 ± 5	81 ± 3
LVEDP (mmHg)	0.8 ± 0.3	1.1 ± 0.9	1.7 ± 0.7	3.0 ± 0.7	1.4 ± 0.3	2.2 ± 0.6
dP/dt max (mmHg/sec)	13093 ± 991	13598 ± 753	12024 ± 1089	7500 ± 1210 <sup>†‡</sup>	12784 ± 1023	11548 ± 782 <sup>§</sup>
dP/dt min (mmHg/sec)	6788 ± 390	6808 ± 682	6212 ± 577	3967 ± 638 <sup>†‡</sup>	6202 ± 744	5752 ± 469 <sup>§</sup>

BW, body weight; TL, tibial length; HW, heart weight; LVW, left ventricular weight; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; Mean ± SEM; \**P*<0.05 vs. WT; †*P*<0.05 vs. dnPI3K; ‡*P*<0.05 vs. WT-DOX; §*P*<0.05 vs. dnPI3K-DOX; ¶*P*<0.05 vs. NRG1-WT-DOX.

**Table 2:** Cardiac hemodynamic measurements of DOX-treated and NRG1-treated dnPI3K mice.

activating alternative survival pathways, such as ERK1/2 and mTORC1.

#### Acknowledgements

This work is supported by American Heart Association Grant-In-Add (Yan X, 10GRNT4710003) and NHLBI (Yan X, HL106098). We thank Acorda Therapeutics Inc. for providing recombinant NRG1 (recombinant human glial growth factor 2 - rhGGF2). We thank Dr. Lewis C. Cantley for his invaluable advice on this work.

#### References

- Falls DL (2003) Neuregulins: functions, forms, and signaling strategies. *Exp Cell Res* 284: 14-30.
- Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2: 127-137.
- Liu FF, Stone JR, Schuldt AJ, Okoshi K, Okoshi MP, et al. (2005) Heterozygous knockout of neuregulin-1 gene in mice exacerbates doxorubicin-induced heart failure. *Am J Physiol Heart Circ Physiol* 289: H660-666.
- Liu X, Gu X, Li Z, Li X, Li H, et al. (2006) Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. *J Am Coll Cardiol* 48: 1438-1447.
- Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, et al. (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344: 783-792.
- Bian Y, Sun M, Silver M, Ho KK, Marchionni MA, et al. (2009) Neuregulin-1 attenuated doxorubicin-induced decrease in cardiac troponins. *Am J Physiol Heart Circ Physiol* 297: H1974-1983.
- Fuller SJ, Sivarajah K, Sugden PH (2008) ErbB receptors, their ligands, and the consequences of their activation and inhibition in the myocardium. *J Mol Cell Cardiol* 44: 831-854.
- Fukazawa R, Miller TA, Kuramochi Y, Frantz S, Kim YD, et al. (2003) Neuregulin-1 protects ventricular myocytes from anthracycline-induced apoptosis via erbB4-dependent activation of PI3-kinase/Akt. *J Mol Cell Cardiol* 35: 1473-1479.
- Baliga RR, Pimental DR, Zhao YY, Simmons WW, Marchionni MA, et al. (1999) NRG-1-induced cardiomyocyte hypertrophy. Role of PI-3-kinase, p70(S6K), and MEK-MAPK-RSK. *Am J Physiol* 277: H2026-2037.
- Cantley LC (2002) The phosphoinositide 3-kinase pathway. *Science* 296: 1655-1657.
- Liu P, Cheng H, Roberts TM, Zhao JJ (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 8: 627-644.
- Shaw RJ, Cantley LC (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* 441: 424-430.
- McMullen JR, Amirahmadi F, Woodcock EA, Schinke-Braun M, Bouwman RD, et al. (2007) Protective effects of exercise and phosphoinositide 3-kinase(p110alpha) signaling in dilated and hypertrophic cardiomyopathy. *Proc Natl Acad Sci U S A* 104: 612-617.
- Oudit GY, Penninger JM (2009) Cardiac regulation by phosphoinositide 3-kinases and PTEN. *Cardiovasc Res* 82: 250-260.
- Lin RC, Weeks KL, Gao XM, Williams RB, Bernardo BC, et al. (2010) PI3K(p110 alpha) protects against myocardial infarction-induced heart failure: identification of PI3K-regulated miRNA and mRNA. *Arterioscler Thromb Vasc Biol* 30: 724-732.
- Liu P, Cheng H, Roberts TM, Zhao JJ (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 8: 627-644.
- Shioi T, Kang PM, Douglas PS, Hampe J, Yballe CM, et al. (2000) The conserved phosphoinositide 3-kinase pathway determines heart size in mice. *EMBO J* 19: 2537-2548.
- Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, et al. (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest* 118: 3065-3074.
- O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, et al. (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 66: 1500-1508.
- Alessi DR (2001) Discovery of PDK1, one of the missing links in insulin signal transduction. *Colworth Medal Lecture. Biochem Soc Trans* 29: 1-14.
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307: 1098-1101.
- Outomuro D, Grana DR, Azzato F, Milei J (2007) Adriamycin-induced myocardial toxicity: new solutions for an old problem? *Int J Cardiol* 117: 6-15.
- Cheng H, Force T (2010) Molecular mechanisms of cardiovascular toxicity of targeted cancer therapeutics. *Circ Res* 106: 21-34.
- Force T, Kerkela R (2008) Cardiotoxicity of the new cancer therapeutics--mechanisms of, and approaches to, the problem. *Drug Discov Today* 13: 778-784.
- Barry E, Alvarez JA, Scully RE, Miller TL, Lipshultz SE (2007) Anthracycline-induced cardiotoxicity: course, pathophysiology, prevention and management. *Expert Opin Pharmacother* 8: 1039-1058.
- Telli ML, Hunt SA, Carlson RW, Guardino AE (2007) Trastuzumab-related cardiotoxicity: calling into question the concept of reversibility. *J Clin Oncol* 25: 3525-3533.
- Yuan TL, Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 27: 5497-5510.
- Sarbassov DD, Ali SM, Sabatini DM (2005) Growing roles for the mTOR pathway. *Curr Opin Cell Biol* 17: 596-603.
- Manning BD, Logsdon MN, Lipovsky AI, Abbott D, Kwiatkowski DJ, et al. (2005) Feedback inhibition of Akt signaling limits the growth of tumors lacking Tsc2. *Genes Dev* 19: 1773-1778.

30. Fruman DA, Meyers RE, Cantley LC (1998) Phosphoinositide kinases. *Annu Rev Biochem* 67: 481-507.
31. Chua YL, Ito Y, Pole JC, Newman S, Chin SF, et al. (2009) The NRG1 gene is frequently silenced by methylation in breast cancers and is a strong candidate for the 8p tumour suppressor gene. *Oncogene* 28: 4041-4052.
32. Tsai MS, Shamon-Taylor LA, Mehmi I, Tang CK, Lupu R (2003) Blockage of heregulin expression inhibits tumorigenicity and metastasis of breast cancer. *Oncogene* 22: 761-768.
33. Aguilar Z, Akita RW, Finn RS, Ramos BL, Pegram MD, et al. (1999) Biologic effects of heregulin/neu differentiation factor on normal and malignant human breast and ovarian epithelial cells. *Oncogene* 18: 6050-6062.
34. Shelton EH, Benjamin FC, Carolyn IS (2003) The EGF receptor family--multiple roles in proliferation, differentiation, and neoplasia with an emphasis on HER4. *Trans Am Clin Climatol Assoc* 114: 315-334.
35. Xu F, Yu Y, Le XF, Boyer C, Mills GB, et al. (1999) The outcome of heregulin-induced activation of ovarian cancer cells depends on the relative levels of HER-2 and 25 HER-3 expression. *Clin Cancer Res* 5: 3653-3660.
36. Harris LN, Yang L, Liotcheva V, Pauli S, Iglehart JD, et al. (2001) Induction of topoisomerase II activity after ErbB2 activation is associated with a differential response to breast cancer chemotherapy. *Clin Cancer Res* 7: 1497-1504.
37. Harris LN, Yang L, Tang C, Yang D, Lupu R (1998) Induction of sensitivity to doxorubicin and etoposide by transfection of MCF-7 breast cancer cells with heregulin beta-2. *Clin Cancer Res* 4: 1005-1012.

**Citation:** Bian Y, Silver M, Onufrak J, Ho KKL, Marchionni MA, et al. (2013) Neuregulin1 Improved Cardiac Function in Doxorubicin-Treated Mice with Cardiomyocyte-Specific over expression of a Dominant-Negative PI3Kp110 $\alpha$ . J Cardiovasc Dis Diagn 1: 120. doi:[10.4172/2329-9517.1000120](https://doi.org/10.4172/2329-9517.1000120)