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Impaired Cardiac Function in a mouse model of Generalised Glucocorticoid Resistance

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Background: Glucocorticoids regulate a broad spectrum of physiologic functions essential for life and exert their actions through their ubiquitously expressed glucocorticoid receptor (GR). The GR interacts with several molecules, including the non-coding RNA growth arrest-specific 5 (Gas5), which binds to the DNA-binding domain of the GR, acting as a decoy glucocorticoid response element (GRE) and competing with DNA GREs for binding to the GR [1]**(Fig.1)**. Therefore, Gas5 decreases the transcriptional activity of the GR and reduces tissue sensitivity to glucocorticoids.

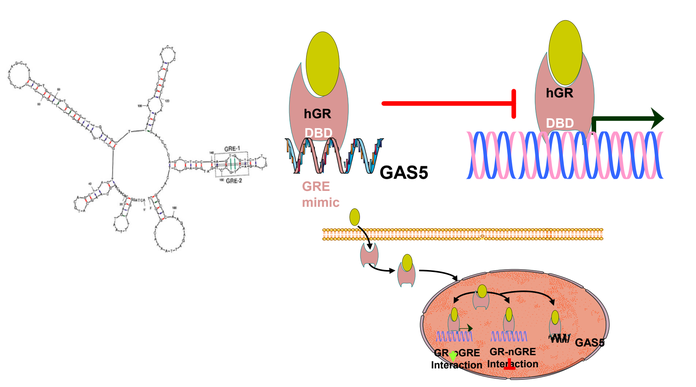

Figure 1: Mechanism of Gas5 interaction with GR.

Objective: To create a mouse model of Generalized Glucocorticoid Resistance (GGR) by inducible overexpression of Gas5 and to investigate its myocardial function.

Methods: For the generation of transgenic mice expressing Gas5, we used the inducible tetracycline system Tet On [2] (**Fig.2**) **,** as it tightly controls expression of the linked transgene upon doxycycline administration. Two transgenic lines expressing the reverse transactivator (rtTA) under hnRNP promoter and the Gas5 under Tet responsive P tight promoter were generated and then crossed to create double transgenic mice (Gas5/rtTA) (**Fig.2**). RNA was isolated from heart tissues after 2 weeks of doxycycline (DOX-) or water (DOX-) administration. Expression of Gas5 was measured with qRT-PCR.

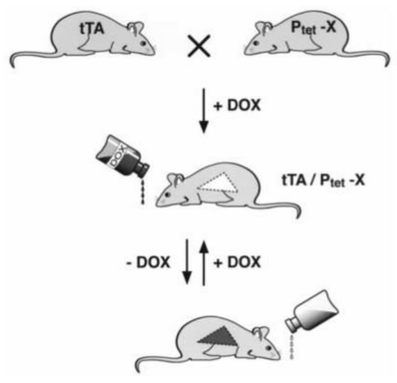

Figure 2:The Tet-On system

Cardiac function was evaluated by echocardiography (13 MHz linear probe, GE) and 24-hour electrocardiography (ECG) in Gas5/rtTA/DOX+ and Gas5/rtTA/DOX- mice (double transgenic mice without doxycycline administration), as well as in the wild-type mice with (WT/DOX+) or without (WT/DOX-) doxycycline administration. Left ventricular (LV) end-diastolic (LVEDD) and end-systolic diameter (LVESD), left ventricular posterior wall thickness at diastole (PWT) and the ratio of LV to PWT (r/h) were determined. The global LV function was evaluated by calculating the percentage of LV fractional shortening (%, FS). The heart rate (HR), and the number of ventricular and supraventricular arrhythmias were measured by 24-hour ECG recordings using surgically placed ECG electrodes that transmitted data to a telemetry ECG receiving system. ECG data were gathered for 5 min every 30 min during the 24-hour ECG recording and analyzed.

Results: Genetic constructs of double transgenic mice inducibly overexpressing Gas5 after doxycycline administration (DOX+) were generated. The induction of overexpression of Gas5 in: Gas5/rtTA mice (2 weeks DOX+;0.78±0.37) compared with i)Gas5/rtTA/DOX-mice (0.14±0.04), ii) single transgenic rtTA/DOX+ mice where the TetOn system is not functional ($0.3*10^{-4}\pm0.5*10^{-5}$), and iii) WT/DOX+ mice ($0.7*10^{-5}\pm0.8*10^{-5}$) was verified in the myocardium (**Fig 3).**

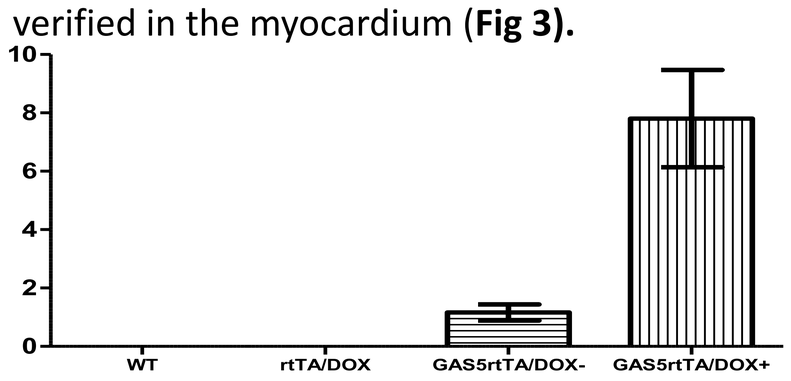

Figure 3:Expression study of Gas5 in cardiac tissues.

Cardiac function (% FS) was significantly decreased in Gas5/rtTA/DOX+ compared to Gas5/rtTA/DOX- (44.6±0.8 vs 48.5±0.4; p=0.003).) but not compared to WT/DOX+ (46.9±0.4, p=0.2 as shown in **Table 1**. The reduction was mainly due to decreased systolic function in Gas5/rtTA/DOX+ (LVESD: 1.77±0.01mm; p=0.05) as shown in **Table 2**, **Fig.4.**

ECG studies did not show differences among the three groups in terms of HR, ECG interval measurements and arrhythmias.

WT/DOX+ (WT) vs GAS5/rtTA/DOX+ (On)			
Table 1	WT	On	p value
	n=7	n=7	
HR	582.85±25.90	598.85±16.68	0.61
EDD(mm)	3.01±0.04	3.24±0.10	0.07
ESD(mm)	1.62±0.02	1.79±0.06	0.04
PWd(mm)	0.78±0.01	0.77±0.01	0.27
PWs(mm)	1.31±0.01	1.27±0.01	0.02
FS (%)	46.09±0.76	44.67±0.59	0.17
r/h	1.92±0.02	2.10±0.06	0.02

Table 1. Echocardiography study measurements in WT/DOX+ (WT) and GAS5/rtTA/DOX+ (On) mice

GAS5/rtTA/DOX+ (On) vs GAS5/rtTA/DOX- (Off)			
Table 2	Off (w/o)	On	p value
	n=5	n=5	
HR	600.40±26.84	596.40±24.06	0.93
EDD(mm)	3.04±0.08	3.20±0.13	0.23
ESD(mm)	1.56±0.04	1.77±0.08	0.05
PWd(mm)	0.81±0.01	0.78±0.01	0.003
PWs(mm)	1.32±0.01	1.28±0.01	0.003
FS (%)	48.45±0.41	44.58±0.78	0.003
r/h	1.88±0.04	2.05±0.07	0.07

Table 2. Echocardiography study measurements in GAS5/rtTA/DOX+ (On) and GAS5/rtTA/DOX- (Off) mice

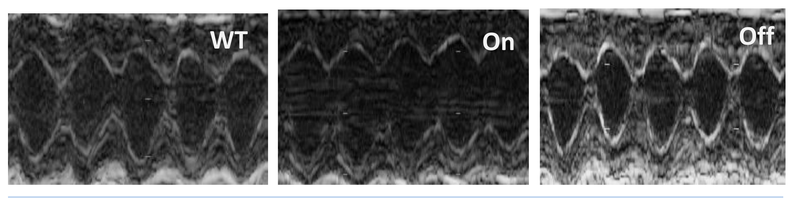

Figure 4. LV M-mode echocardiography images from WT/DOX+ (WT), GAS5/rtTA/DOX+ (On) and GAS5/rtTA/DOX- (Off) mice

Conclusions: We created a mouse model of GGR and demonstrated impaired LV function. Ongoing studies aim to investigate the molecular mechanisms through which Generalized Glucocorticoid Resistance affects myocardial function.

References

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2.Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci U S A 1992; 89, 5547-5551.

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