Rapid Communication

Myocardial stress remodelling after regional infarction is independent of glycogen synthase kinase-3 inactivation

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Phosphorylation and inactivation of glycogen synthase kinase 3 (GSK-3) is observed in the failing heart induced by chronic pharmacological stress and aortic banding. Constitutive kinase activity attenuates pathological remodelling, suggesting an obligatory role in stress signalling. However, this has been challenged by recent data whereby conditional GSK-3β deletion has been shown to protect against post-infarct remodelling. Here, we set out to determine the chronic remodelling response to infarction in hearts of GSK-3α [Ala21/9] knockin (KI) mice encoding constitutively active GSK-3 isoforms. At 4 weeks after infarction there were significant increases in normalised heart weight and left ventricular (LV) muscle volume compared to sham in both KI and wild type animals. This was associated with an increase in LV cavity dimensions and remote LV wall thickness. Hypertrophy in both genotypes resulted in marked contractile impairment on both invasive and non-invasive interrogation. Increased phosphorylation of GSK-3β, but not GSK-3α, was demonstrated at 1 week after infarction and remained elevated at 4 weeks compared to sham-treated hearts. In conclusion, GSK-3β phosphorylation and inactivation occurs with, but is not an obligatory signalling event in, chronic post-infarct remodelling in the mouse heart. This highlights the heterogeneity of pathological hypertrophy and the divergent role of GSK-3 signalling in chronic myocardial stress.

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1. Introduction

Glycogen synthase kinase-3 (GSK-3) signalling is implicated in pathological stress remodelling in the heart. Two isoforms are expressed equally within cardiomyocytes, GSK-3α and GSK-3β, but it is inactivation of the latter through N-terminal Serβ-phosphorylation which is considered crucial in pathological hypertrophy. GSK-3β phosphorylation is demonstrated with aortic banding or treatment with prohypertrophic agonists such as isoproterenol, phenylephrine and endothelin-1 [1–3]. Conversely, hypertrophy induced by these interventions is attenuated through expression of inactivation-resistant GSK-3β[Serβ] or overexpression of wild-type GSK-3β [1–4].

Chronic remodelling after regional infarction shares many of the pathological traits induced by other experimental hypertrophy protocols, including cardiomyocyte hypertrophy, interstitial fibrosis and foetal gene reactivation [5,6]. Infarction is the predominant substrate for heart failure in clinical practice [7], and arguably represents a more pathophysiologically relevant platform for study, despite its relative complexity. To what extent common signalling pathways exist between these models, however, is unclear. In particular, there is a relative paucity of data regarding GSK-3 activity, with a paradoxical bias towards the clinical literature. Human tissue obtained at transplantation or at the time of ventricular assist device implantation for end-stage heart failure, for example, confirms increased GSK-3β phosphorylation, but a more heterogeneous signalling response to mechanical off-loading [8,9].

Recently, data have been published in a mouse with inducible cardiomyocyte-specific GSK-3β deletion (conditional knockout) [10]. Eight weeks after permanent ligation of the proximal left anterior descending artery, KO hearts demonstrated exaggerated hypertrophy, associated with a paradoxical attenuation in ventricular dilatation and contractile dysfunction. Examination of remote myocardium confirmed a proliferative cardiomyocyte response with hypertrophy and a reduction in apoptosis [10]. Paradoxically therefore, pathological remodelling in this setting may be attenuated by the absence of GSK-3 kinase activity.

In order to clarify the role of GSK-3, we set out to examine post-infarction remodelling in GSK-3α [Ala21/9] knockin (KI) mice expressing constitutively active forms of the kinase within the heart.

2. Methods

2.1. GSK-3α [Ala21/9] KI mice

KI mice were kindly provided by Professor Alessi (University of Dundee, UK). The targeting strategy and consequent effect on PKB/Akt-GSK3-GS signalling axis have been described previously [1,11,12]. All experiments were performed in accordance with UK Home Office regulations.

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2.2. LAD ligation

Experiments were undertaken in weight-matched (25–30 g) adult male mice. Animals were subjected to permanent left anterior descending (LAD) artery ligation or sham procedure, as previously described [13]. Risk area was determined by intravenous delivery of Evan’s blue solution (0.5 ml, 2%). Hearts were then explanted, fixed (10% paraformaldehyde) and sectioned (700 μm) for planimetry, as described [1,13]. Infarct size was calculated morphologically as the mean percentage scar circumference relative to total LV circumference across all heart cross-sections.

2.3. Cardiac remodelling

In recovery experiments, hearts of surviving mice were examined at 4 weeks after LAD ligation or sham procedure by echocardiography, pressure-volume interrogation and morphometry of explanted hearts, as described [1,13]. Infarct size was calculated morphologically as the mean percentage scar circumference relative to total LV circumference across all heart cross-sections.

2.4. Protein chemistry

In parallel experiments in WT animals, hearts were rapidly excised at set time points after surgery for protein interrogation. Total and phosphoserine-Ser21/9 levels of GSK-3 were estimated by SDS-PAGE and western blotting of whole heart homogenates, as previously described [1,12]. Insulin-treated hearts were used as positive controls for GSK-3 inactivation, as previously [1,12].

2.5. Statistics

Data are presented as means ± SEM. Scatter plot data were subjected to linear regression analysis. Survival was analysed by the Kaplan–Meier method with a log-rank test for differences between groups. Comparisons between groups were assessed for significance by one-way or two-way analysis of variance (ANOVA), as appropriate. When significant differences were detected, individual mean values were compared by Bonferroni’s post hoc test. A P-value less than 0.05 was considered significant.

3. Results

3.1. Determination of risk area

Initial experiments set out to exclude potential bias effects of variant epicardial coronary anatomy between genotypes. Reassuringly, no differences in area at risk (AAR) were detectable between KI, WT or outbred C57BL/6 mice (AAR 45.4 ± 6.8%, 40.3 ± 5.1% and 39.6 ± 3.4%, respectively, n = 8/group, p = ns) (Fig. 1A).

3.2. Survival and GSK-3α/β phosphorylation after MI

Survival rates after infarction were identical between genotypes (Fig. 1B) despite heart homogenates in WT animals revealing inactivating phosphorylation of GSK-3β, but not GSK-3α, at the end of the first and fourth post-operative weeks (Fig. 1D).

3.3. Chronic ventricular remodelling

Morphological assessment of WT hearts at 4 weeks after surgery confirmed significant hypertrophy in infarct- vs. sham-treated animals, with increases in normalised heart weight (HW/BW 7.5 ± 0.5 vs. 5.3 ± 0.3 mg/g, n = 6/group, p < 0.05) and calculated left ventricular muscle volume (LVV 169.2 ± 21.9 vs. 99.9 ± 7.1 μl/g, n = 6/group, p < 0.05) (Table 1). Mean LV measurements taken from 700 μm-thick sections at the mid-level of the papillary muscle confirmed scar thinning (317 ± 56 vs. 1275 ± 62 μm sham, p < 0.05) and remote hypertrophy of viable myocardium (1810 ± 87 vs. 1327 ± 64 μm, p < 0.05) (Table 1). Infarct remodelling at 4 weeks was associated with significant ventricular dilatation and impairment of contractility, both from invasive and non-invasive interrogation (Table 1, Fig. 1E). Importantly, hearts of KI animals demonstrated similar infarct sizes (Table 1, Fig. 1C), morphological and physiological changes following LAD ligation, without evidence of protection or, conversely, potentiation of injury and pathological remodelling.

4. Discussion

The major finding of this study is that constitutive GSK-3α/β activity is unable to prevent chronic stress remodelling in murine hearts following regional infarction. This contrasts directly with protection against chronic β-adrenergic stimulation or pressure-overload demonstrated previously using this genetic approach [1,2]. Together, these data highlight the mechanistic heterogeneity of pathological hypertrophy, and the likely redundancy of GSK-3 in more complex models.

As a direct inhibitory regulator of a number of key transcription and translational processes, GSK-3 is proposed to be a strategic point of signalling integration in both developmental cardiac growth and pathological remodeling. Attention has largely focused on GSK-3α, with expression of inactivation-resistant isoforms robustly protective against both pharmacological and pressure-induced hypertrophy [1–4]. GSK-3α activity may counterbalance some of these beneficial effects during pathological growth, with enhanced fibrosis and blunted cardiomyocyte proliferation [2,14]. However the biological significance of this remains unclear, since targeted studies in isoform-specific and dual isoform knockin mice suggest phenotype-dominance of the GSK-3α isoform during stress [2].

The recent demonstration that inducible GSK-3β deletion protects murine hearts from chronic remodelling after myocardial infarction, questions the obligatory role of GSK-3β inactivation in pathological hypertrophy and highlights the likely model-dependence of this process [10]. However, GSK-3 function may extend beyond that of its kinase activity, since it can act to coordinate protein complexes which maybe relevant to cell death [15]. Hence conditional knockout and constitutively-active knockin mice are not antitheses. Furthermore, using the same mouse line we have previously shown that pathological hypertrophy in response to isoproterenol is attenuated [1]. Together with the findings here, this suggests that the role of GSK-3 may differ by physiological stress, in keeping with findings in the GSK-3β cardiomyocyte specific conditional KO mouse [10].

Accepted limitations of our genetic model include potential non-selective cellular consequences distinct from Ser21/9 mutation, and unexpected systemic responses with ubiquitous mutant kinase expression. Experiments in isoform-specific KI animals were not performed, since double KI animals revealed no overt stress-phenotype compared to WT, but this limitation is also acknowledged.

In summary, although GSK-3β phosphorylation occurs in the failing mouse heart after regional infarction, GSK-3β inactivation does not appear to be an obligatory signalling event in pathological remodelling.

Disclosure

Conflicts of interest: none

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Fig. 1. LAD ligation experiments. (A) Area at risk (AAR) distal to the LAD ligature was determined in wild type (WT), knockin (KI) and outbred C57BL/6 mice by Evan's Blue staining (0.5 ml, 2%) and planimetry of sectioned (700 μm) hearts. (B) In recovery experiments, no difference was seen in survival between genotypes following infarction. (C) Representative examples of heart cross-sections (700 μm) at 4 weeks after myocardial infarction (MI) or sham procedure. (D) Representative immunoblots of phosphorylated and total GSK-3 protein levels in WT heart homogenates taken at 1 day, 1 week and 1 month after LAD ligation (MI) or sham. Insulin-treated hearts are used as positive controls for GSK-3 phosphorylation. Quantitative data for GSK-3\(\beta\) phosphorylation are also shown as a ratio of phospho/total protein and represent the mean±SEM of 4 independent experiments; *p<0.05 vs. sham at indicated time points. (E) Echocardiography was performed at 4-weeks under isoflurane anaesthesia (1–2%). FS=fractional shortening, LVIDd/s=left ventricular internal dimension (diastole/systole). N=8/group, *p<0.05 vs. sham.
Table 1
Morphology and pressure–volume interrogation. Hearts were subjected to P–V interrogation under isoflurane anaesthesia 4 weeks after infarction or sham procedure. Morphometry was performed on fixed heart sections (700 μm) at the level of the mid-papillary muscle. N = 6/group, *p<0.05 vs sham (within genotype). HR = heart rate, SV = stroke volume, CO = cardiac output, EF = ejection fraction, dP/dtmax = maximum first derivative of pressure, dP/dtmin = minimum first derivative of pressure, ESV = end-systolic pressure, ESP = end-systolic volume, Ea = arterial elastance, SW = stroke work, EDV = end-diastolic volume, τ(Weiss) = isovolumic time constant of relaxation.

|                      | Sham     | MI     | Sham     | MI     |
|----------------------|----------|--------|----------|--------|
| **Morphology**       |          |        |          |        |
| HW/BW (mg/g)         | 5.3±0.3  | 7.5±0.5* | 5.9±0.4  | 7.8±0.6* |
| LV muscle volume (μl3)| 99.9±7.1 | 169±21.9* | 119.1±5.7 | 180±19.0* |
| LW/BW (mg/g)         | 6.9±0.5  | 8.4±1.1 | 6.8±0.7  | 9.7±0.8  |
| Scar thickness (μm)  | 1275±62  | 317±56  | 1300±62  | 293±92*  |
| Remote LV thickness (μm) | 1327±64   | 1810±87* | 1362±44  | 163±63*  |
| Infarct size (% of LV circumference) | N/A     | 37±6   | N/A     | 41±9     |
| **P–V Analysis**     |          |        |          |        |
| **Systole**          |          |        |          |        |
| HR (bpm)             | 634±12   | 587±10 | 610±17   | 608±21  |
| SV (μl)              | 16.9±1.0 | 13.1±1* | 15.9±0.6 | 12.9±1.2 |
| CO (ml/min)          | 107±0.6  | 77.4±0.4* | 97.3±0.4  | 7.3±0.6  |
| EF (%)               | 69±3     | 37.4±4* | 74.4     | 44.4±4*  |
| dP/dtmax (mm Hg/s)   | 6824±309 | 5531±483 | 6464±272 | 598±550  |
| ESP (mm Hg)          | 86.8±2.2 | 86.2±60 | 85.8±2.4 | 91.8±4.4 |
| ESV (μl)             | 7.6±0.8  | 22.8±2.3* | 6.0±1.0  | 16.6±2.0* |
| SW                   | 801±70   | 548±74  | 709±74   | 497±84   |
| Ea (mm Hg/μl)        | 5.4±0.4  | 7.3±0.8  | 6.1±0.4  | 7.7±0.9  |
| **Diastole**         |          |        |          |        |
| dP/dtmin (mm Hg/s)   | -3306±134 | -2935±791 | -2956±220 | -3304±548 |
| ESP (mm Hg)          | 8.0±0.4  | 11.7±1.1* | 8.3±0.4  | 12.9±1.7* |
| EDV (μl)             | 24.5±1.6 | 35.8±2.1* | 21.9±1.4 | 29.4±2.4* |
| T (Weiss) (ms)       | 6.2±0.2  | 8.2±0.3* | 6.4±0.2  | 8.4±0.7*  |

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