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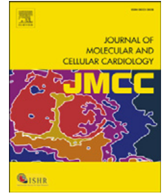

Original article

Focal adhesion kinase governs cardiac concentric hypertrophic growth by activating the AKT and mTOR pathways

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ABSTRACT

The heart responds to sustained overload by hypertrophic growth in which the myocytes distinctly thicken or elongate on increases in systolic or diastolic stress. Though potentially adaptive, hypertrophy itself may predispose to cardiac dysfunction in pathological settings. The mechanisms underlying the diverse morphology and outcomes of hypertrophy are uncertain. Here we used a focal adhesion kinase (FAK) cardiac-specific transgenic mice model (FAK-Tg) to explore the function of this non-receptor tyrosine kinase on the regulation of myocyte growth. FAK-Tg mice displayed a phenocopy of concentric cardiac hypertrophy, reflecting the relative thickening of the individual myocytes. Moreover, FAK-Tg mice showed structural, functional and molecular features of a compensated hypertrophic growth, and preserved responses to chronic pressure overload. Mechanistically, FAK overexpression resulted in enhanced myocardial FAK activity, which was proven by treatment with a selective FAK inhibitor to be required for the cardiac hypertrophy in this model. Our results indicate that upregulation of FAK does not affect the activity of Src/ERK1/2 pathway, but stimulated signaling by a cascade that encompasses PI3K, AKT, mTOR, S6K and rpS6. Moreover, inhibition of the mTOR complex by rapamycin extinguished the cardiac hypertrophy of the transgenic FAK mice. These findings uncover a unique role for FAK in regulating the signaling mechanisms that governs the selective myocyte growth in width, likely controlling the activity of PI3K/AKT/mTOR pathway, and suggest that FAK activation could be important for the adaptive response to increases in cardiac afterload. This article is part of a Special Issue entitled “Local Signaling in Myocytes”.

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1. Introduction

Under sustained hemodynamic overload, the heart initiates a process of hypertrophic growth, whereby the myocytes, in addition to becoming enlarged, change their shape in patterns that are specific to the inciting mechanical stress [1,2]. Accordingly, the width of myocytes increases disproportionately (as compared with their length) in response to enhanced cardiac afterload, resulting in a concentric type of hypertrophy, whereas sustained increases in the preload result in proportional growth in the width and length of individual myocytes and, therefore, in eccentric hypertrophy. Though adaptive in nature, these patterns of hypertrophy may be themselves deleterious, contributing to maladaptive remodeling in conditions such as hypertension, valvular heart disease and myocardial infarction [3]. In this case, cardiac chambers undergo progressive dilation and wall thinning, reflecting the excessive lengthening without further change in the width of the myocytes, a feature that appears to be consistently deleterious, because it associates with progression toward heart failure [4]. From a molecular perspective, it is now generally accepted that distinct signaling pathways are responsible for the different patterns and outcomes of hypertrophy [5], but the understanding of such pathways is still incomplete. Therefore, more knowledge about the signaling mechanisms underlying the various patterns of hypertrophy and outcomes should have considerable clinical consequences and may be seminal for the development of novel and better strategies to prevent and treat cardiac diseases.

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase and a scaffold protein lying downstream of signaling by integrin, growth factor and neuro-hormonal receptors, as a regulator of fundamental cellular functions, such as adhesion, growth, migration and survival

Abbreviations: FAK, focal adhesion kinase; FAK-Tg, cardiac-specific FAK transgenic mice model; FAKi, pharmacological FAK inhibitor (PF 573228); RP, rapamycin; TAC, thoracic aortic constriction.

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[6]. In differentiated cardiac myocytes, FAK is highly expressed and is implicated in the hypertrophic response to biomechanical stress and agonists of adrenergic, angiotensin and endothelin receptors [7–10]. Besides, expression and activity of FAK are enhanced in the myocytes of human and murine models of cardiac hypertrophy [11–13]. A causal link between signaling by FAK and cardiac hypertrophy was strengthened by the observation that depletion of global myocardial FAK by RNA interference prevents the hypertrophic growth induced by pressure overload in mice [12]. In addition, the relevance of FAK signaling for the hypertrophic responses of cardiac myocytes was examined in cardiac specific FAK conditional knockout mice by two different groups [14,15]. This approach, however, has led to controversies because of differences of the phenotypic effects of FAK depletion in the myocytes. For instance, although both lineages of FAK knockout mice showed no change in basal structure and function, one lineage developed eccentric hypertrophy [14] while the other [15] showed mild to moderate attenuation of reactive hypertrophy in response to aortic constriction. Thus, the role of FAK in regulating the myocyte hypertrophic growth remains inconclusive.

To determine whether FAK signaling pathway functions in the determination of cardiac hypertrophy we used a cardiac myocyte-specific transgenic strategy. We observed that moderate increases in FAK expression induces functional, structural and molecular features of a concentric, but compensated type of cardiac hypertrophy. Consistent with this phenotype, myocytes isolated from FAK-Tg mice had an increased cross sectional area, but no change in the length, as compared to myocytes of wild type mice. Our studies also provide evidence that the pro-hypertrophic effects of FAK are likely mediated via AKT and mTOR signaling.

2. Methods

An expanded Methods section is available in the Supplementary data.

2.1. Materials and antibodies

Anti-FAK, anti-GAPDH, anti-Src, anti-ERK1/2, anti-phospho-ERK1/2, anti-S6K and anti-phospho-S6K were from Santa Cruz Biotechnology. Anti-AKT, anti-phospho-AKT-473, anti-PI3K, anti-mTOR, anti-TSC2 and anti-phospho-TSC2-1462 were from Cell Signaling. Anti-phospho-FAK-397, anti-phospho-FAK-925 and anti-phospho-Src418 were from BioSource International. Anti-rpS6 and anti-phospho-rpS6 were from Bethyl. FAK-full length was purchased from Invitrogen-PV383, Rapamycin was from LC Laboratories and pharmacological FAK inhibitor (PF 573228) was from Tocris Bioscience. Oligonucleotides were from IDT.

2.2. Generation of FAK-Tg mice

FAK-Tg mice were generated by lentiviral vector injection in freshly fertilized oocytes from FVB mice. Briefly, the ubiquitin promoter from FUW lentiviral vector [16] was replaced by the 1.6-kb murine α-MHC promoter [17] at PacI and BamHI site to generate the FαMHCW vector. FAK cDNA was amplified by PCR from pRcCMV-FAK using the forward (5′ AACGATCCGGCGCGCCATGGAGCAGAAGCT3′) and reverse (5′ TTGGATCGGGCGCGCCTCAGTGTGGCCGTG 3′) primers. The PCR product was digested with AscI and was cloned into FαMHCW at the AscI site to generate FαMHCFAKW vector. Lentiviruses were packed using standard protocol of calcium phosphate transfection in 293T cells. Genomic DNA from mice tail tissue was prepared by standard proteinase K digestion and isopropanol precipitation procedure. Transgenic mice were genotyped by PCR using the forward primer for the 3′ portion of α-MHC promoter (5′ TGGGAGAGCCATAGGCTAC 3′) and the reverse for the 5′ portion of FAK cDNA (5′ GATGAAAGACCTTTAATACTCG 3′).

2.3. Model of chronic pressure overload

Aortic banding was performed by transverse aortic constriction as detailed in Supplementary data.

2.4. Echocardiography

2D M-mode echocardiography was performed with a 15-MHz probe connected to a Philips HD11.

2.5. Western blotting and immunoprecipitation

Generation of protein samples from tissue, along with Western blotting are described in Supplementary data.

2.6. Real time PCR

Technique and oligonucleotides used in the reactions are described in the online Supplementary data.

2.7. Isolation of adult cardiac myocytes

Adult mice ventricular myocytes were isolated using a collagenase digestion method, as described in the online Supplementary data.

2.8. Histology and staining

Hearts were collected and stained as indicated in Supplementary data.

2.9. Transmission electron microscopy

Fragments of the mouse left ventricle were fixed in 2.5% glutaraldehyde (Electron Microscope Science, Hatfield, PA, USA), followed by postfixation in 1% osmium tetroxide and embedded in Epon 812 (Electron Microscope Science). Ultrathin sections were double stained by 2% uranyl acetate and 0.5% lead citrate and were examined in a Leo 906 transmission electron microscope.

2.10. Statistical analysis

Data are presented as mean ± SEM. Comparisons between multiple samples were performed by one-way ANOVA analysis with Bonferroni post hoc corrections. Comparisons between FAK-Tg and control littermate mice were performed by unpaired *t*-test with Welch's correction. P values <0.05 indicated statistical significance.

3. Results

3.1. Generation of mice overexpressing FAK in the heart

We produced three independent, stable, lines of FAK-Tg mice by injecting one-cell FVB/N mouse embryos with a lentivirus that harbors FAK under the control of the cardiac α-myosin heavy chain (*α-MyHC*) (Fig. 1A). Founders (2 males, 1 female) were crossed into the FVB/N background. Using real time PCR with WRE specific primers we established that the lineages display 1 to 2 transgene copies (FAK-Tg1: 2 copies, FAK-Tg2: 2 copies, FAK-Tg3: 1 copy). The α-MyHC-FAK transgene was transmitted through the progeny with Mendelian frequency and the animals were followed through at least 1 year without manifesting any abnormalities. All experiments were performed in heterozygous animals.

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