Real-time monitoring of hypertrophy in HL-1 cardiomyocytes by impedance measurements reveals different modes of growth

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Abstract Hypertrophic growth is a response of the heart to increased mechanical load or physiological stress. Thereby, cardiomyocytes grow in length and/or width to maintain cardiac pump function. Major signaling pathways involved in cardiomyocyte growth and remodeling have been identified during recent years including calcineurin–NFAT and PI3K–Akt signaling. Modulation of these pathways is of certain interest for therapeutic treatment of cardiac hypertrophy. However, quantification and characterization of hypertrophy in response to different stimuli or modulators is difficult. This study aims to test different read-out systems for detection and quantification of

differences in hypertrophic growth in response to prohypertrophic stimuli. Real-time impedance measurements allowed the detection of distinct differences in hypertrophic growth in response to endothelin, norepinephrine, phenylephrine or BIO, which were not observable by other methods such as flow cytometry. Endothelin treatment induced a rapid and strong peak in the impedance signal concomitant with a massive reorientation of the actin cytoskeleton. Changes in expression of hypertrophy-associated genes were detected and stabilization of β -catenin was identified as a common response to all hypertrophic stimuli used in this study. Hypertrophic growth was blocked by the PI3K/mTOR inhibitor PI-103.

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Introduction

The mammalian heart is an organ that dynamically adapts to changes in workload with the aim to maintain heart function and adequate blood flow in the body. A typical response to prolonged hypertensive pressure overload or other stress factors is myocardial hypertrophy. Since cardiomyocytes are terminally differentiated non-dividing cells, the pump force of the heart only can be enhanced by lateral and/or longitudinal growth of the cells. Independent of the stimulus the initial hypertrophic growth in most cases is a compensatory adaption to augment contractility of the heart and is reversible. However, persistent mechanical load or pressure finally results in irreversible hypertrophy with development of heart failure.

During recent years, different signaling pathways promoting hypertrophic growth of cardiomyocytes have been identified. Mechanical stress signals are initiated by mechanosensitive ion channels, which affect intracellular Ca^{2+} -signaling (Stiber et al. 2009). Release of Ca^{2+} from the sarcoplasmic reticulum activates calmodulin and in consequence the serine/threonine phosphatase calcineurin. Subsequent calcineurin-mediated dephosphorylation of NFAT (nuclear factor of activated T cells) induces its nuclear translocation where NFAT activates transcription of target genes such as β -myosin heavy chain and brain natriuretic peptide (BNP) (Molkentin et al. 1998). Activation of calcium/calmodulin-dependent protein kinase II (CaMKII) induces phosphorylation of class II histone deacetylases (HDACs) and their nuclear export thus preventing their interaction with the transcription factor MEF2 (myocyte enhancer factor 2), which then reprograms cardiac gene expression (Lu et al. 2000; Zhang et al. 2002). Activation of G-protein-coupled receptors (GPCRs) by intrinsic factors such as endothelin (ET1), norepinephrine or angiotensin II, which are released in response to increased mechanical stress, results in induction of the PI3K/Akt signaling cascade. Glycogen-synthase-kinase-3 β (GSK-3 β) is then phosphorylated by Akt and inactivated. Since NFAT is a target of GSK-3 β , it is no

longer phosphorylated and thus can translocate to the nucleus thereby reprogramming gene expression (Haq et al. 2000). Vice versa, activation of GSK-3 β suppresses cardiac hypertrophy (Antos et al. 2002).

Multiple other pathways such as MAP-kinase and TNF α signaling have been shown to contribute to myocardial hypertrophy. Moreover, an essential role for β -catenin in physiological and pathological myocardial hypertrophy was shown in cell culture and animal models (Baurand et al. 2007). Stabilization of β -catenin was observed in cardiomyocytes in response to hypertrophic stimuli in an Akt- and GSK-3 β -dependent mechanism (Haq et al. 2003). This state-of-affairs can either occur by Akt-dependent direct phosphorylation of β -catenin (Fang et al. 2007) or by Akt-dependent inhibition of GSK-3 β . Independent of the mechanism, stabilization and nuclear translocation of β -catenin finally contributes to changes in gene transcription. All the changes in the cellular phenotype in pathological myocardial hypertrophy are accompanied by the re-expression of genes active during embryonic development of the heart.

The signaling pathways contributing to cardiac hypertrophy are potential targets for therapeutic treatment. Better understanding of the effects of hypertrophic substances would benefit from simple quantitative measurement of hypertrophic growth in cell culture. We addressed this problem using label-free, non-invasive impedance assays in a real-time mode. This highly sensitive system allowed detection of distinct differences when hypertrophic growth of murine HL-1 cardiomyocytes was induced by ET1, norepinephrine or phenylephrine. These differences were not detectable by flow cytometric analyses or microscopy but had impact on gene expression and actin cytoskeleton organization. All hypertrophy-inducing substances tested in this study resulted in a stabilization of β -catenin. This means that macroscopic hypertrophic growth in HL-1 cells can be differentiated depending on the stimulus and this may be relevant for therapeutic treatment.

Materials and methods

Cell culture

HL-1 cells (obtained from Prof. William Claycomb, LSU Health, School of Medicine, New Orleans, LA,

USA) were grown in Claycomb medium (Sigma, Taufkirchen, Germany) supplemented with 2 mM GlutaMAXTM (Invitrogen; Karlsruhe, Germany), 100 U/ml penicillin (PAA), 100 µg/ml streptomycin (PAA, Pasching, Austria) and 10 % (v/v) FCS (Sigma; F2442 Lot#058K8426) in the presence or absence of norepinephrine on gelatine-fibronectin [0.02 % (w/v) gelatine; 5 mg/ml fibronectin] (Sigma) pre-coated plates (Claycomb et al. 1998). Cells were seeded and treated between 7 and 9 a.m. to reduce circadian variations. Cells were kept serum-free for 24 h before stimulation with the GSK-3β inhibitor BIO ((2′Z,3′E)-6-bromindirubin-3′-oxime) (Calbiochem, Schwalbach, Germany), endothelin-1 (ET1) (Sigma), norepinephrine (NE) (Sigma) or phenylephrine (PE) (Sigma) for 12–48 h. As a solvent control 3 µM ascorbic acid was used. The PI3K/mTOR inhibitor PI-103 was dissolved in DMSO as a 10 mM stock solution.

Impedance measurements

The impedance-based measurements were performed with Real-Time Cell Analyzer (RTCA) DP instrument (xCelligence System from Roche Applied Science, Mannheim, Germany). The RTCA with its three 16-well plate (E-plate) holders was placed into an incubator at 37 °C and 5 % CO₂. Into each well, precoated with gelatine/fibronectin, after pre-testing 25,000 cells were plated in 200 µl serum-free Claycomb-medium supplemented with GlutaMAXTM, penicillin and streptomycin (see above), a cell number that is in the range given by other studies (Lamore et al. 2013; Xi et al. 2011). The cell index (CI), was measured continuously every 10 min at a frequency of 10 kHz. At each time point three measurements are combined to a mean CI value. The CI is a self-calibrated value derived from the ratio of measured impedances according to the following formula: $CI = (Z_i - Z_0)/15 \Omega$. Z_i are impedances at the different time points and Z_0 is background resistance before seeding of cells on the plate, meaning the resistance of only medium. Instrument adjustments are specified by the xCELLigence system and CI values were calculated by the xCELLigence System software package. After 24 h when cells had settled and attached on the plates and the cell index remained constant, the cells were stimulated with either 0.5 µM BIO (Sinha et al. 2005), 100 nM ET1 (Cullingford et al. 2008; Haq et al.

2003), 10 µM NE (Koshman et al. 2010) or 100 µM PE (Haq et al. 2003). Concentrations of hypertrophy inducers were chosen according to values given in the literature and by pre-testing of different concentrations of each substance. Cell indices (CIs) were normalized at this point before further recording of the CI for 48 h. CI values of four independent measurements were exported to Excel and mean values were calculated.

Flow cytometry

HL-1 cells were cultivated and stimulated for 48 h with BIO, ET1, NE or PE as described above. Microscopic images were taken on a Motic AE21 inverse microscope (Microscope World, Carlsbad, CA, USA). For subsequent flow cytometry (CyFlow Space, Sysmex Partec, Görlitz, Germany), cells were trypsinized for 5 min and washed with PBS. Cell size and shape were analyzed using the forward (FSC) and sideward (SSC) scat mode. Values were normalized to the FSC or SSC values of unstimulated, solution-treated HL-1 cells.

Immunofluorescence microscopy

HL-1 cells were seeded on pre-coated cover slides in Claycomb medium without FCS. After 24 h cells were stimulated for 1 or 3 h. Cells were washed once with PBS and subsequently fixed with 4 % para-formaldehyde/4 % (w/v) saccharose for 15 min at RT. Cells were permeabilized with 0.4 % (v/v) Triton X-100 for 8 min and subsequently blocked for 20 min in blocking solution [1 % (v/v) FCS (Biochrom GmbH, Berlin, Germany), 1 % (w/v) fish gelatine (Sigma), 1 % (w/v) BSA (PAA)]. Alexa Fluor594 phalloidin (Invitrogen) was diluted 1:20 together with 1 µg/ml DAPI (Sigma) in blocking solution and cells were incubated for 30 min to stain the actin cytoskeleton and cell nuclei, respectively. After washing in PBS, cover slides were mounted on microscope slides using Mowiol (Roth, Karlsruhe, Germany) mixed with 25 mg/ml DABCO (Roth). Images were taken on an Axio Observer.Z1 Apotom (Zeiss, Jena, Germany).

Western blotting

HL-1 cells were stimulated for different periods of time with BIO, ET1, NE or PE as described above.

Cells were lysed with ice-cold lysis buffer (50 mM Tris/HCl pH 7.5, 120 mM NaCl, 0.25 % (v/v) Triton X-100, 10 % (v/v) glycerol, 1 mM MgCl₂ and Complete protease inhibitor mix without EDTA (Roche Applied Science) for 10 min at 4 °C. Equal amounts of cell lysates were separated by SDS-PAGE and blotted onto PVDF membrane. Detection of β -catenin and GAPDH was performed as described previously (Weiske et al. 2007). Anti- β -catenin (clone-14) (BD Bioscience, Heidelberg, Germany) and anti-GAPDH (Merck Millipore, Darmstadt, Germany) antibodies were diluted 0.1 and 1 μ g/ml, respectively. Chemiluminescence signals were detected and quantified on a SynGene imager and β -catenin signals were normalized to the GAPDH signal used as a loading control.

Quantitative RT-PCR

Cells were stimulated for 24 h as described above. Total RNA was isolated using the NucleoSpin RNA II Kit (Macherey–Nagel, Düren, Germany) according to the manufacturer's recommendations. Reverse transcription was performed with High-capacity cDNA Reverse Transcription Kit (Invitrogen Life Technologies, Karlsruhe, Germany) from 1 μ g RNA. Real-time PCR was performed with the Maxima Probe/ROX qPCR Master Mix (Fermentas, St. Leon-Rot, Germany) using TaqMan probes ACTC1: Applied Biosystems Mm01333821_m1 (Life Technologies); Nppa: Ax-051029-00-0100, c-myc: Ax-040813-00-0100 (Thermo Scientific, Dreieich, Germany) all FAM dye-labeled. Mouse β -actin (ACTB: Applied Biosystems 4352341E-0909011 (Life Technologies) was VIC-MGB-labeled and served as endogenous control and was not affected under experimental conditions. Each PCR was set up in duplicates and threshold cycle (C_t) values of the target genes were normalized to the endogenous control. Differential expression was calculated according to the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

For analysis of statistical significance paired *t*-test was used. Number of experiments is indicated in the respective figure legends.

Results

HL-1 cells as an in vitro cardiomyocyte hypertrophy model

HL-1 mouse cardiac muscle cells originally isolated by William Claycomb were chosen as cellular system for quantitative measurement of cardiomyocyte hypertrophy. HL-1 cells have been characterized to proliferate in culture and maintain their differentiated cardiac phenotype as shown by morphological and electrophysiological properties as well as gene expression profiles (Claycomb et al. 1998).

When HL-1 cells were cultivated in the standard Claycomb-medium containing 100 μ M norepinephrine (NE), addition of ET1 did not induce a detectable increase in size or change in morphology. We assumed that HL-1 cells under these conditions already are hypertrophic and thus do not show a significant further response. Therefore, we decided to grow HL-1 cells in supplemented Claycomb-medium but without NE. After 2 weeks cultivation in NE-depleted medium, already microscopic investigation revealed an apparent decrease in cell size and especially intracellular granules (not shown) with cells stopping to beat. Since changes in cell size are difficult to quantify under a standard light microscope, forward-scattering (FSC) and sideward-scattering (SSC) measurements in a flow cytometer were performed (Dowling et al. 2010; Rosner et al. 2009). Consistent with the microscopic observations, SSC signals were reduced in NE-depleted cells compared to HL-1 cells grown in NE-containing medium. FSC signals were only marginally reduced after depletion of NE. When NE was re-added to the medium, after 2 days HL-1 cells started to beat again and cell size increased as detected by flow cytometry (Suppl. Fig. 1a). NE-starved cells did not show any change in the expression of α -actin 1 (Actc1), a marker for differentiated cardiac muscle cells, in qRT-PCR (Suppl. Fig. 1b). Therefore, we decided to use HL-1 cells grown in NE-depleted medium for our further experiments.

Real-time impedance measurements detect different modes of hypertrophic growth

In a next set of experiments we incubated NE-depleted HL-1 cells with 100 nM endothelin-1 (ET1), 10 μ M NE, 50 μ M phenylephrine (PE) as known inducers of

cardiomyocyte hypertrophy. In addition, we treated cells with 500 nM or 1 μ M BIO, a GSK-3 β inhibitor, which induces stabilization of β -catenin and thus should promote hypertrophic growth. HL-1 cells were seeded in serum-free medium to stop proliferation and after 24 h cells were stimulated with the different inducers for further 48 h. In light microscopic evaluation, ET1-treated cells showed increased numbers of intracellular granules and a change in cell size, which was difficult to quantify with a standard cell culture microscope. NE- and PE-treated cells also revealed increased granularity with granules appearing larger in size compared to ET1-treatment. Addition of 500 nM BIO resulted in less granularity, and cell shape appeared more elongated in contrast to ET1, NE and PE treated cells (Suppl. Fig. 2). Flow cytometric analyses confirmed an increase in cell size and granularity in the forward-scatter (FSC) and sideward-scatter (SSC) mode of measurement, respectively (Suppl. Fig. 3). Although flow cytometry clearly showed an increase in overall cell size in response to hypertrophic stimuli, a disadvantage of this method is that cells have to be trypsinized to release them from the bottom of the plates and in consequence cells round up. An increase in volume then is correlated with hypertrophic growth.

Therefore, we decided to apply impedance measurements using a real time cell analyzer system (XCelligence RTCA DP) to quantify hypertrophy of adherent HL-1 cells. This system is highly suitable to assess cell viability, proliferation and migration (Roshan-Moniri et al. 2015). The increase in size associated with hypertrophic growth was expected to coincide with a rise in the impedance signal when HL-1 cells are grown on E-plates lined with tiny gold electrodes at the bottom of the well. For efficient adhesion of the cells, E-plates were pre-coated with gelatine/fibronectin according to normal growth conditions (Claycomb et al. 1998). After 24 h in serum-free medium, cells stopped proliferation and the impedance signal reached a plateau. When HL-1 cells were stimulated with ET1, NE, PE or BIO, a significant increase in the impedance signals was detectable over time (Fig. 1a) resulting in an increase of relative cell indices (Fig. 1b). To confirm, that the rise in impedance correlates with hypertrophic growth and not with cell proliferation, cell numbers were counted after trypsinization and did not show significant differences (Suppl. Fig. 4). Impedance

measurements revealed a comparable induction of hypertrophic growth for ET1, NE and PE, whereas BIO (500 nM) was less efficient. Interestingly, in contrast to endpoint measurements, real-time impedance recordings especially within the first hours after induction of hypertrophy revealed major differences between ET1-induced hypertrophy and the other stimuli. ET-1 treatment resulted in a rapid increase of the impedance signal with a peak at 2–3 h after addition of ET1. The impedance signal then dropped again within 5 h. Thereafter, the cell index steadily increased at a slow rate until it finally reaches a plateau after 48 h.

Fig. 1 Impedimetric measurement of hypertrophic growth of HL-1 cells. **a** 2.5×10^4 cells were plated on gelatine/fibronectin-coated wells of E-plates in serum-free Claycomb-medium (–NE) and grown for 24 h. At this time point the cell index was normalized and DMSO as solvent control or ET1 (100 nM), NE (10 μ M), PE (100 μ M) or BIO (1 μ M) were added. The cell index was recorded every 10 min for 48 h. **b** The graph represents relative cell indices at time point 48 h ($n = 4$). * $p < 0.05$

Endothelin-1 induces changes in the HL-1 actin cytoskeleton

Since cells did not proliferate, the observed rapid increase in the cell index after ET1-addition was assumed to be the result of a change in the actin cytoskeleton. To investigate this in more detail HL-1 cells were stained with Alexa Fluor594 phalloidin at different time points after stimulation of hypertrophy and analyzed by immunofluorescence microscopy. Interestingly, in ET1-treated cells a marked formation of actin stress fibers was detectable 1 and 3 h after stimulation (Fig. 2a). By contrast, when cells were stimulated with the other hypertrophy inducers, stress fiber formation was not observed comparably. Even when cells were treated with NE (Fig. 2a), PE, BIO (Suppl. Fig. 5) for 48 h, no stress fibers were detectable similar to untreated cells. Western blot analyses did not show differences in the expression levels of β -actin (Fig. 2b). Taken together, the observed ET1-specific mode of hypertrophic growth corresponds to an extensive formation of stress fibers not detectable for the other hypertrophy inducers.

Differences in c-myc and ANP/Nppa expression in ET-1, NE, PE or BIO-treated cells

Next we investigated if the differences detected in the mode of hypertrophic growth also have a correlation at the gene expression level. The known hypertrophy associated markers c-myc (Xiao et al. 2001) and ANP (atrial natriuretic peptide) were chosen for qRT-PCR analyses. HL-1 cells were stimulated for 24 h with ET1, NE, PE or BIO and subsequently total RNA was isolated for cDNA synthesis and quantitative PCR. ET-1 treatment increased both the expression of the hypertrophy-associated markers c-myc (Xiao et al. 2001) and ANP (Nishikimi et al. 2006). BIO surprisingly reduced c-myc expression and had no significant effect on ANP expression. NE-treatment upregulated c-myc expression similar to ET1 but repressed ANP expression. Finally, PE also increased c-myc expression, but to a lower level than ET1 and had no effect on ANP levels (Fig. 3a, b).

Stabilization of β -catenin is a common effect in hypertrophic HL-1 cells

Inhibition of GSK-3 β with BIO resulted in β -catenin accumulation in the cytosol and nucleus. In our HL-1

model, treatment with BIO promoted hypertrophic growth of HL-1 cells. Analysis of β -catenin levels in HL-1 cells after stimulation with BIO, ET1, PE and NE revealed that increased amounts of β -catenin were detectable in all cells independent of the stimulant, with BIO showing the most prominent effect (Fig. 3c). From these observations we conclude, that stabilization of β -catenin is a phenomenon common in hypertrophic HL-1 cells independent of the stimulus.

The PI3K/Akt/mTOR inhibitor PI-103 attenuates hypertrophic growth

Finally, we wanted to test if the observed hypertrophic effect is induced via the PI3K/Akt/mTOR pathway and if hypertrophic growth can be modulated by a specific inhibitor. As a proof of principle substance, we used the PI3K/Akt/mTOR axis inhibitor PI-103. Akt is important for physiological hypertrophy but long-term overexpression led to pathological hypertrophy (Shiojima et al. 2005). Hypertrophic growth of HL-1 cells in response to ET1 and NE was indeed attenuated in the presence of 100 nM PI-103 as shown by flow cytometric measurements (Fig. 4a, b). When PI-103 was added to HL-1 cells in impedance recordings, we again detected the early ET1-induced rapid increase in CI values. Consistent with cytometric measurements a dose-dependent reduction of the CI was observed in PI-103 treated HL-1 cells over time (Fig. 4c).

Discussion

Multiple intracellular signaling pathways have been identified, which are involved in regulation of cardiac hypertrophy (Harvey and Leinwand 2011; Heineke and Molkentin 2006). These pathways provide options for pharmacological targeting and subsequent therapeutic treatment. However, screening for inhibitors or modulators of cardiomyocyte hypertrophy is hampered by the difficult determination of hypertrophic growth (types). Here, we investigated hypertrophic growth characteristics of HL-1 cardiomyocytes in response to different stimuli and a pharmacological inhibitor in vitro using impedance measurements, immunofluorescence and qRT-PCR. As previously shown the principle of impedance measurement is suitable to detect hypertrophic growth (Yang et al.

Fig. 2 ET1 induces a rapid reorganization of the actin cytoskeleton in HL-1 cells. **a** HL-1 cells were treated with ET1 and NE and subsequently fixed at the indicated time points for staining of actin filaments with phalloidin-Alexa594. Immunofluorescence microscopy was performed on a Zeiss

2007). Further analysis of the cells by immunofluorescence and qRT-PCR characterize the type of hypertrophy in more detail. Using the HL-1 cell line with its very stable properties avoids variability

Axio Observer.Z1 Apotom. **b** Western blot analyses of whole cell lysates did not show differences in the amount of β -actin 1 or 3 h after addition of ET1 or NE, respectively. Densitometric quantification of the Western blot signals ($n = 2$)

problems immanent to primary cardiomyocytes. The advantage of impedimetric analyses is that hypertrophic growth of adherent cells can be measured in real-time and in a quantitative manner in contrast to

Fig. 3 Expression of c-myc (**a**) and ANP/Nppa (**b**) in response to ET1, NE, PE or BIO treatment. HL-1 cells were treated as described above and 24 h after stimulation RNA was isolated for qRT-PCR. The data show mean values $\pm$ SEM ($n = 8$).

* $p < 0.05$; ** $p < 0.005$. **c** Quantification of β -catenin by Western blotting and chemiluminescence imaging 12, 18, and 24 h after stimulation with ET1, NE, PE or BIO. Data show mean values $\pm$ SEM ($n = 7$). * $p < 0.05$; ** $p < 0.005$

endpoint measurements e.g. by flow cytometry. In our assay system, cells were grown in serum-free medium until they stopped to proliferate before hypertrophy was induced. This is a prerequisite to exclude, that the increase of the impedance signal is an effect of cell proliferation.

The opportunity to perform real-time monitoring allowed us to detect distinct differences in the hypertrophic response to ET1 and NE or PE. The ET1-specific rapid peak in the impedance signal suggested that a rapid redistribution of the cytoskeleton occurred. Phalloidin-staining revealed the formation of actin fibers as a rapid response to ET1 treatment. There is evidence that Rho/ROCK signaling is activated in response to ET1 in cardiomyocytes (Kuwahara et al. 1999) inducing the translocation of myocardin-related transcription factor (MRTF)-A into the nucleus, where MRTF-A acts as a SRF co-activator (Kuwahara et al. 2010). Another difference in hypertrophic growth is the mRNA profile of cells stimulated with ET1, NE or PE. An increase in Nppa and c-myc expression is a defined marker for hypertrophic growth. Only ET1 stimulation correlates with enhanced expression levels of both Nppa and c-myc. NE and PE showed a different expression pattern in

line with the results from impedance measurement and phalloidin-staining suggesting that there exist different types of hypertrophic growth. Our experiments moreover showed, that stabilization of β -catenin appears to be a common effect in hypertrophic HL-1 cells independent of the stimulating factor. Stabilization of β -catenin similarly has been shown in response to ET1 and PE treatment of neonatal rat ventricular myocytes and in hearts of TAC (thoracic aortic constriction) rats (Haque et al. 2003).

Moreover, impedance measurement allowed to detect that the PI3K/mTOR inhibitor PI-103 attenuates ET1-induced hypertrophy of HL-1 cells consistent with the known role of PI3K/Akt/mTOR signaling cascade in cardiac hypertrophy (for review Aoyagi and Matsui 2011; Lee et al. 2007). A dose-dependence was clearly detectable. Taken together, HL-1 cells exhibited typical cardiomyocyte hypertrophic behavior in respect to growth and to effects of a PI3K/Akt/mTOR inhibitor and β -catenin accumulation but revealed a discrimination of hypertrophic subtypes in respect to actin cytoskeleton organization and mRNA expression levels depending on the stimuli.

In summary, differences in the modes of hypertrophic growth of HL-1 cells to specific stimuli can

Fig. 4 Hypertrophic growth is attenuated by the PI3K/Akt/mTOR inhibitor PI-103. **a** Hypertrophy in HL-1 cells was induced with ET1 or NE for 48 h in the presence or absence of 100 nM PI-103 and detected by flow cytometry. **b** Results of

five independent experiments are summarized. * $p < 0.005$. **c** Impedimetric measurement allowed detection of a dose-dependent inhibition of hypertrophic growth by PI-103 as shown by an exemplary real-time recording of cell indices (CI) for 48 h

easily be detected by real-time impedance measurements in a label-free, quantitative online measurement as observed for the rapid ET-1-induced changes in the actin cytoskeleton. For our proof-of-principle experiments we used the RTCA DP instrument. The RTCA

Cardio instrument may be more sensitive and provide higher resolution for future studies especially when beating activity of cardiomyocytes is of interest (Xi et al. 2011). It cannot be excluded that cardiomyocytes may respond to electrical impedance, however, to our

knowledge this has not been observed up to now. The sensitive detection of specific responses to hypertrophic stimuli and its consequences on the cell behavior might be useful for the characterization of stimulus-specific anti-hypertrophic drug candidates.

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