Acta Biomaterialia 58 (2017) 323–336

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Engineered myocardium model to study the roles of HIF-1α and HIF1A-AS1 in paracrine-only signaling under pathological level oxidative stress

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ARTICLE INFO

Article history:
Received 22 February 2017
Received in revised form 11 May 2017
Accepted 15 June 2017
Available online 16 June 2017

Keywords:
Oxidative stress
Tissue engineering
Hypoxia inducible factor
Cardiomyocyte
Endothelial cell

ABSTRACT

Studying heart tissue is critical for understanding and developing treatments for cardiovascular diseases. In this work, we fabricated precisely controlled and biomimetic engineered model tissues to study how cell-cell and cell-matrix interactions influence myocardial cell survival upon exposure to pathological level oxidative stress. Specifically, the interactions of endothelial cells (ECs) and cardiomyocytes (CMs), and the role of hypoxia inducible factor-1α (HIF-1α), with its novel alternative regulator, HIF-1α antisense RNA1 (HIF1A-AS1), in these interactions were investigated. We encapsulated CMs in photo-crosslinkable, biomimetic hydrogels with or without ECs, then exposed to oxidative stress followed by normoxia. With precisely controlled microenvironment provided by the model tissues, cell-cell interactions were restricted to be solely through the secreted factors. CM survival after oxidative stress was significantly improved, in the presence of ECs, when cells were in the model tissues that were functionalized with cell attachment motifs. Importantly, the cardioprotective effect of ECs was reduced when HIF-1α expression was knocked down suggesting that HIF-1α is involved in cardioprotection from oxidative damage, provided through secreted factors conferred by the ECs. Using model tissues, we showed that cell survival increased with increased cell-cell communication and enhanced cell-matrix interactions. In addition, whole genome transcriptome analysis showed, for the first time to our knowledge, a possible role for HIF1A-AS1 in oxidative regulation of HIF-1α. We showed that although HIF1A-AS1 knockdown helps CM survival, its effect is overridden by CM-EC bidirectional interactions as we showed that the conditioned media taken from the CM-EC co-cultures improved CM survival, regardless of HIF1A-AS1 expression.

Statement of Significance

Cardiovascular diseases, most of which are associated with oxidative stress, is the most common cause of death worldwide. Thus, understanding the molecular events as well as the role of intercellular communication under oxidative stress is upmost importance in its prevention. In this study we used 3D engineered tissue models to investigate the role of HIF-1α and its regulation in EC-mediated cardioprotection. We showed that EC-mediated protection is only possible when there is a bidirectional crosstalk between ECs and CMs even without physical cell-cell contact. In addition, this protective effect is at least partially related to cell-ECM interactions and HIF-1α, which is regulated by HIF1A-AS1 under oxidative stress.

1. Introduction

Myocardial infarction (MI), or heart attack, is one of the most common causes of death worldwide [1]. The immediate action taken after a heart attack is to reoxygenate the infarct tissue by restoring blood flow. However, the sudden flow of oxygen-rich blood to the ischemic area induces oxidative stress and often results in further cell death causing reperfusion injury (RI) [1–3], which can lead to the death of the patient [4]. Several mechanisms and metabolic pathways are suggested to be involved in this phenomenon [5] however, a detailed understanding of the progression and prevention of RI is yet to be discovered.

A possible means of treatment during and after RI is to use bioactive factors that would protect the cells from the damage

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http://dx.doi.org/10.1016/j.actbio.2017.06.023
1742-7061/

caused by the oxidative stress that occurs once the blood flow is restored. Heart tissue has endogenous protective processes in the early stages of MI as well as its aftermath. A candidate mediator of cardioprotection is endothelial cell (EC)-cardiomyocyte (CM) communication via paracrine factors such as nitric oxide (NO), endothelin 1 (EDN1), angiopoietin-II, and prostaglandin I_2 [6]. One potential mediator of this myocardial cellular communication during and after MI is hypoxia inducible factor-1 (HIF-1). HIF-1 is a heterodimeric transcription factor (comprised of HIF-1α and HIF-1β subunits) upstream of over 100 genes [7–9]. Due to its oxygen dependent post-translational regulation, the role of the HIF-1α subunit in protection from ischemic diseases under hypoxic conditions has been studied extensively. However, even though there is evidence that oxidative stress can stabilize HIF-1α [10,11], its regulation under oxidative stress is poorly studied. In addition, the results of many of these studies examining the exact role of HIF-1 or HIF-1α during MI vary widely [12–17]. Some studies showed that HIF-1 enhances myocardial cell survival [12,17], while others showed that it enhances cell death through increased apoptosis [14,18] and increases the infarct size [13]. One of the major reasons for these contradictory outcomes is the complexity of the in vivo environment. In addition, there are often discordances between animal studies and clinical trials due to differences in human and animal physiology and pathology [19]. On the other hand, cell culture studies are an oversimplification of the native phenomena where the cell-cell and cell-extracellular matrix (ECM) interactions are almost entirely misrepresented. As an alternative approach, it is now possible through tissue engineering techniques to fabricate model tissues *in vitro* with better-controlled parameters and using human cells [20–22]. Using such tissue engineered model myocardial tissues with defined cellular composition and microenvironment would be a very powerful research approach to study the role of HIF-1α and the paracrine factors regulated by HIF-1α under RI mimicking oxidative stress conditions. Moreover, it would serve as a platform to study potential therapeutics for RI treatment.

In this study, we developed 3-dimensional (3D) tissue engineered myocardial model tissues using primary neonatal rat CMs and human induced pluripotent stem cell (hiPSC)-derived ECs (iECs). We studied the effect of EC-CM interactions solely through secreted factors as well as cell-ECM interactions on cell survival under oxidative stress conditions mimicking the early onset of RI. We used rat origin CMs and human origin ECs, which allowed us to investigate the changes in their mRNA expression separately yet allowing a successful intercellular communication owing to the high level of protein homology between rats and humans in paracrine factors such as vascular endothelial growth factor (VEGF) [23]. Using these model tissues, we showed that EC-CM interactions, specifically mediated through EC-driven HIF-1α expression, improve cell survival under oxidative stress. We also showed evidence, for the first time in literature, of an alternate possible means of HIF-1α regulation under oxidative stress through HIF-1α antisense RNA1 (HIF1A-AS1), which could have an important role in the cardioprotective effect of EC-CM crosstalk.

2. Materials and methods

An expanded Methods section is available in the Online Data Supplement. All animal experiments were performed according to the guidelines of Institutional Animal Care and Use Committee (IACUC) of University of Notre Dame.

2.1. Cell culture and HIF-1α knockdown

2-day-old Sprague-Dawley rats (Charles River Laboratories) were sacrificed by decapitation and the hearts were immediately excised following the Institutional Animal Care and Use Committee (IACUC) guidelines of the University of Notre Dame, which has an approved Assurance of Compliance on file with the National Institutes of Health, Office of Laboratory Animal Welfare. The hearts were rinsed in ice-cold Hank's Balanced Salt Solution (HBSS, Gibco) immediately and the respective CMs were isolated and cultured following well established protocols [24].

The hiPSCs (line DiPS SevA1016) derived from fibroblasts were differentiated to iECs following a recently established protocol [25]. Briefly, the hiPSCs were cultured on Geltrex (Invitrogen) coated tissue culture flasks with mTeSR1 (StemCell Technologies) and, to induce differentiation, the culture media was switched to N2B27 medium (1:1 mixture of DMEM:F12 (1:1) with Glutamax and Neurobasal media supplemented with N2 and B27) (Life Technologies) supplemented with a glycogen synthase kinase 3β (GSK3β) inhibitor, CHIR (Stemgent) and bone morphogenic protein 4 (BMP4) (R&D Systems). The media was replaced with StemPro-34 SFM medium (Life Technologies) (supplemented with 200 ng/mL VEGF (PeproTech) and 2 μM forskolin (Sigma-Aldrich)) after three days. The media was renewed the following day and at the end of day six, the cells were sorted using magnetic assisted cell sorting (MACS) (autoMACSpro, Miltenyi Biotec, Harvard University) against vascular endothelial cadherin (VE-CAD). The purity of the cell population after sorting was determined using fluorescence assisted cell sorting (FACS) against VE-CAD (MACSQuant, Miltenyi Biotec, Harvard University). The collected cells were then cultured on fibronectin coated tissue culture dishes in endothelial growth media-2 (EGM-2). The endothelial phenotype of the iECs was confirmed using quantitative polymerase chain reaction (qPCR), immunostaining, and tube formation assay, and compared with human umbilical cord vein endothelial cells (HUVECs). In some experiments, when CMs and ECs were required to be monitored separately in the culture, ECs were marked by using Cell Tracker Blue (Invitrogen).

The HIF-1α knockdown was introduced by small hairpin RNA (shRNA) targeting of HUVECs and iECs. The change in HIF-1α expression was then examined on mRNA and protein levels using qPCR and enzyme-linked immunosorbent assay (ELISA), respectively.

2.2. Conventional cell culture and model tissue fabrication

For conventional, 2-dimensional (2D) cell culture experiments, the CMs (1×10^5 cells/well) were seeded with or without HIF-1α shRNA knockdown or control iECs or HUVECs (2.5×10^4 cells/well) into regular 96 well tissue culture plates ($n \geq 6$).

The 3D model tissues were prepared by encapsulating CMs alone (9×10^5 cells/construct), iECs or HUVECs alone (2×10^5 cells/construct) or as co-culture (9×10^5 CMs and 2×10^5 iECs or HUVECs per construct) in Arginine-Glycine-Aspartic acid (RGD) (Bachem) conjugated or pure poly(ethylene glycol) diacrylate (JenKem) (PEG-RGD) hydrogels ($n \geq 6$). The cell densities in 3D tissue constructs were calculated by estimating the number of cell layers throughout the volume of the constructs (one cell layer was estimated to be approximately 12 μm thick, giving 8.3 layers per construct) and each layer was aimed to have approximately equal cell numbers as in 2D culture samples.

2.3. Oxidative stress treatment and determination of cell survival and apoptosis

Conventional cell cultures and the engineered tissue constructs were cultured in minimal media (DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin) for all experiments unless stated otherwise. Both the cell cultures and model tissues were allowed to pre-incubate for 3 days before being exposed to oxidative stress. The oxidative stress was applied