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Bioluminescence imaging of cardiomyogenic and vascular differentiation of cardiac and subcutaneous adipose tissue-derived progenitor cells in fibrin patches in a myocardium infarct model

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ABSTRACT

Background: Adipose tissue-derived progenitor cells (ATDPCs) isolated from human cardiac adipose tissue are useful for cardiac regeneration in rodent models. These cells do not express cardiac troponin I (cTnI) and only express low levels of PECAM-1 when cultured under standard conditions. The purpose of the present study was to evaluate changes in cTnI and PECAM-1 gene expression in cardiac ATDPCs following their delivery through a fibrin patch to a murine model of myocardial infarction using a non-invasive bioluminescence imaging procedure.
Methods and results: Cardiac and subcutaneous ATDPCs were doubly transduced with lentiviral vectors for the expression of chimerical bioluminescent–fluorescent reporters driven by constitutively active and tissue-specific promoters (cardiac and endothelial for cTnI and PECAM-1, respectively). Labeled cells mixed with fibrin were applied as a 3-D fibrin patch over the infarcted tissue. Both cell types exhibited *de novo* expression of cTnI, though the levels were remarkably higher in cardiac ATDPCs. Endothelial differentiation was similar in both ATDPCs, though cardiac cells induced vascularization more effectively. The imaging results were corroborated by standard techniques, validating the use of bioluminescence imaging for *in vivo* analysis of tissue repair strategies. Accordingly, ATDPC treatment translated into detectable functional and morphological improvements in heart function.
Conclusions: Both ATDPCs differentiate to the endothelial lineage at a similar level, cardiac ATDPCs differentiated more readily to the cardiomyogenic lineage than subcutaneous ATDPCs. Non-invasive bioluminescence imaging was a useful tool for real time monitoring of gene expression changes in implanted ATDPCs that could facilitate the development of procedures for tissue repair.

1. Introduction

The goal of tissue engineering (TE) is the generation of a functional tissue replacement using biological and synthetic materials, often in combination with cells and other biochemical factors. One of the major challenges of TE is the restoration of cardiac function after myocardial infarction (MI). Cumulative evidence indicates that TE has only been capable of modest improvements to cardiac function. Thus, novel cell sources, scaffolds, and biochemical factors with the potential to repair injured tissue are needed. *In vivo* analysis of these elements and their interactions requires a great amount of resources and time. Thus, agile analytical procedures for *in vivo* evaluation have the potential to facilitate the development of TE strategies [1–3].

We used non-invasive bioluminescence imaging (BLI) to monitor the behavior of cells seeded in a biomaterial implanted in a mouse model of MI. Due to the capacity of visible light photons to transverse living tissues, BLI allows the distribution, proliferation, and differentiation of luciferase-expressing cells to be monitored in real time in living tissues. By using tissue-specific promoters to regulate the expression of luciferase reporters introduced into living cells, changes in promoter activity translate into measurable changes in photon fluxes that correlate with transcriptional activity. Cardiac (troponin I) and endothelial (PECAM-1) specific promoters were used in the present study to regulate the expression of chimerical luciferase-fluorescent protein reporters.

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This approach allowed us to analyze the cardio-regenerative potential of two related cell populations from adipose tissue.

Human adipose tissue-derived progenitor cells of cardiac origin (cardiac ATDPCs) have inherent cardiac and endothelial differentiation potential, exerting a beneficial histopathological and functional effect upon intramyocardial transplantation in experimental models of MI in rodents [4].

In the present work, we embedded cardiac and subcutaneous ATDPCs in a natural fibrin scaffold as a cell support and transplanted this construct into the ischemic myocardium of infarcted mice in order to compare their gene expression and proliferative behavior. The implanted cells were permanently labeled by transduction with lentiviral vectors for the expression of chimeric photoproteins with two types of activities: bioluminescence for non-invasive monitoring and fluorescence for cell enrichment and histological analysis. Comparison of the cardiomyogenic and endothelial differentiation capacity of ATDPC cell types using the in vivo BLI procedure indicates that cardiac ATDPCs are better cardiomyogenic precursors. Moreover, postmortem validation of BLI results with more standard procedures shows that this is a convenient and sensitive strategy for non-invasive monitoring of changes in gene expression associated with myocardium repair.

2. Methods

2.1. Isolation and culture of cardiac and subcutaneous ATDPCs

Cells were isolated from the epicardiac adipose tissue (cardiac) and fat tissue above the sternum (subcutaneous) of patients undergoing cardiac surgery. Informed consent was obtained from all subjects, and the study protocol conformed to the principles outlined in the Declaration of Helsinki. Biopsy samples were processed and cells isolated as described previously [5]. Adhered cells were cultured to subconfluence under standard conditions.

2.2. Flow cytometry

Immunophenotypical characterization of cardiac and subcutaneous ATDPCs was performed as described previously [4,6].

2.3. Generation of luciferase-fluorescent protein reporters regulated by specific human cardiac troponin I promoter (hcTnIp) and specific human PECAM-1 promoter (hPECAM-1p)

A pLox:PLuc:GFP lentiviral vector containing a fusion reporter comprising PLuc and green fluorescent protein (GFP), was obtained by PCR amplification and standard cloning procedures using the PLuc and GFP genes from the pGL4.10:PLuc (Promega Corporation, Madison, WI) and pEGFP-N1 plasmids (Clontech Lab., Palo Alto, CA). The 340-bp human cTnIp promoter (hcTnIp) was PCR amplified from genomic DNA using 5′-TCCTTGTGTG AGGGAGTGG-3′ and 5′-GGGTGACCTTCAGGGTCC-3′ as described previously [5] and cloned into the pCR2.11 vector (Invitrogen, Paisley, UK). The promoter sequence was removed from the pCR2.11 vector and cloned into the pLox:PLuc:GFP lentivirus vector to obtain Plox:hcTnIp:PLuc:GFP. Human PECAM-1 promoter (hPECAM-1p) was kindly provided by Dr. Carmelo Bernabéu (Centro de Investigaciones Biológicas CSIC, Madrid, Spain) and cloned into pLox:PLuc:GFP to obtain Plox:hPECAM-1p:PLuc:GFP.

The constitutively expressed reporter, the CMV:RLuc:RFP:ttk lentiviral vector containing a trifunctional chimeric construct comprising the RLuc reporter gene, monomeric red fluorescent protein (RFP), and a truncated version of the herpes simplex virus thymidine kinase gene sr39tk (ttk) under transcriptional control of CMV, was a kind gift from Professor S.S. Gambhir (Dept. Radiology, Stanford University, US).

2.4. Lentiviral particle production and ATDPC transduction

Lentiviral production was performed as described [7–9]. ATDPCs were transduced using CMV:hRLuc:RFP:ttk concentrated lentiviral stock (2×10^6 transduction units/mL, MOI = 21) for 48 h. The highest 11% of RFP-expressing cells were selected by FACS. Sorted cells were transduced again with either concentrated lentiviral Plox:hcTnIp:PLuc:GFP or Plox:hPECAM-1p:PLuc:GFP stocks (2×10^6 transduction units/mL, MOI = 21) for 48 h to obtain double-transduced cells: hcTnIp:PLuc·GFP/CMV:RLuc:RFP:ttk-ATDPCs and hPECAM-1p:PLuc:GFP/CMV:RLuc:RFP:ttk-ATDPCs.

2.5. RNA extraction and real-time PCR

Total RNA was extracted from each mouse heart 3 weeks after MI using the RNAeasy mini kit (Qiagen, Dusseldorf, Germany) according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed using the Revertaid First Strand cDNA Synthesis Kit (Fermentas, St. Leon Rot, Germany). FAM-labeled primers/probes were human specific. Details of the quantitative real-time RT-PCR protocol are provided in the Supplemental Material online.

2.6. Myocardial infarction model

The study was performed using 97 female SCID mice (20 to 25 g; Charles River Laboratories Inc.). Myocardial infarction was induced as described previously [4]. Briefly, the animals were intubated and anesthetized with a mixture of O_2/isoflurane and mechanically ventilated. The heart was exposed and the left anterior descending (LAD) coronary artery permanently occluded with an intramural stitch (7–0 silk suture). Sham-operated animals were prepared in a similar manner except without occluding the LAD coronary artery. Mice were then randomly distributed into six experimental groups: control MI, control MI with implanted fibrin patch, sham implanted cell-loaded fibrin patch (cardiac or subcutaneous ATDPCs), and MI implanted cell-loaded fibrin patch (cardiac or subcutaneous ATDPCs).

All procedures have the approval of the Animal Care Committee of the Research Centre and the Government of Catalonia.

2.7. Development and delivery of fibrin patch

To produce the fibrin patch, Tissucol duo Baxter fibrin adhesive was used. Eight microliters of Tissucol solution (fibrinogen 90 mg/mL) were mixed with 1.5×10^6 transduced cells or culture medium. Then, 8 μL of thrombin solution (500 IU/mL) were added for jellification. Fibrin patches loaded with cells were cultured under standard conditions with supplemented α-MEM (10% FBS, 1% P/S, 1% L-glutamine) for 24 h. Cell-loaded and non-loaded fibrin patches were then implanted after MI induction, covering the injured tissue using synthetic surgical glue (Glubran®2) in the healthy myocardium. Fibrin patches were also implanted in sham-operated animals.

Three weeks after implantation, the hearts were arrested in diastole using arrest solution [4], excised, fixed, cryopreserved in 30% sucrose in PBS, embedded in OCT (Sakura), and snap-frozen in liquid nitrogen-cooled isopentane.

2.8. Non-invasive BLI of luciferase activity

For *in vivo* BLI, anesthetized mice bearing a fibrin patch seeded with luciferase reporter-expressing cells were intraperitoneally injected with 150 μL of luciferin (16.7 mg/mL in physiological serum) (Caliper, Hopkinton, MA) to image PLuc activity. The mice were injected intravenously (tail vein) with 25 μL of benzyl coelenterazine (hCTZ) (1 mg/mL in 50/50 propylene glycol/ethanol) (Nanolight Technology, Pinetop, AZ) diluted in 125 μL of water to image RLuc activity. PLuc and RLuc activities were measured in consecutive days. Mice were monitored during a 3-week period at the indicated times. Photons recorded in images were quantified and analyzed using the Wasabi image analysis software (Hamamatsu Photonics).

2.9. Echocardiography

Mouse cardiac function was assessed by transthoracic echocardiography. An ultrasound system (iE33 Echocardiography System, Philips) equipped with a 15-7io MHz linear-array transducer was used to take measurements at baseline (1 day pre-infarction), 1 day post-infarction, and 2 and 3 week post-cell transplantation. The investigators were blinded to the treatment groups. Images were obtained in B-Mode and M-Mode in the parasternal long-axis view. Functional parameters were measured over five consecutive cardiac cycles and calculated using standard methods [10,11]. Left ventricular end-diastolic diameter (LVDd), LV end-systolic diameter (LVDs), LV end-diastolic volume (LVEDV), and LV end-systolic volume (LVESV) were quantified. Left ventricular ejection fraction (LVEF) was calculated as (LVEDV − LVESV)/LVEDV] × 100 [12].

2.10. Morphometry

Mouse hearts were transversally sliced in two segments: apex and base. Eight serial cryosections (spaced 100-μm apart) from the apex segment were stained with Masson's trichrome and morphometric parameters determined using image analysis software (ImageJ, NIH). The infarct size was calculated as: percentage of mean scar area/total LV wall surface × 100. To evaluate infarct thickness, wall thickness was measured at the thinnest and border zones of the infarction. Three measurements per section were performed to determine posterior wall thickness. The mean value of six 200-μm sections was calculated for the thickness parameters. All sections were photographed using a Leica Stereoscope (Leica TL RCI) and a blind analysis performed.

2.11. Immunohistochemistry

Mouse heart cryosections were incubated with primary antibodies against CD31 (0.8 μg/mL) (Abcam), cTnI (2 μg/mL) (Abcam), or phospho-histone 3 (phospho-H3) (2 μg/mL) (CellSignaling). Sections were also incubated with antibodies against RFP and GFP (5 μg/mL) (Abcam) to enhance the detection of transduced cells. Secondary antibodies conjugated with Cy2, DyLight 549, and Cy5 (1 μg/mL) (Jackson ImmunoResearch) were used for detection. Nuclei were counterstained with Hoechst 33342 and the results analyzed using a Leica TCS SP2 laser confocal microscope.