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Flow cytometry analysis of mouse islet cell expression of heparan sulfate (HS), heparan sulfate proteoglycans (HSPGs) and heparanase (HPSE)

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We use this protocol and it's working

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Abstract

Isolated mouse islets were dispersed into single cells using Accutase (Millipore; 250 µl/500 islets. 40,000 islet cells were transferred to individual wells of a 96 well culture plate (CELLSTAR) for staining and flow cytometry analysis or for culture. For intracellular staining, isolated islet cells were fixed and permeabilised using BD Fix/Perm (BD Biosciences) . After a blocking step, the cells were stained with primary antibodies (anti-mouse collagen type XVIII (Col18), anti-mouse CD138 (anti-syndecan-1 (SDC1), anti-mouse CD44, anti-human HS (10E4) or HP3/17 anti-human heparanase (HPSE)) and incubated with fluorescent secondary antibodies. Events were collected using a BD LSR Fortessa flow cytometer with BD FACS DIVA software (version 8). Data was analysed using FlowJo software (version10.0.7, TreeStar Inc.).

Guidelines

10E4 anti-heparan sulfate (HS) mAb identifies highly sulfated HS localised in beta cells but does not identify the less sulfated HS in alpha cells.

Reference:

Theodoraki A, Hu Y, Poopalasundaram S et al (2015) Mol Cell Endocrinol 399: 296-310.

Before start

Materials:

1. Prepare:

(i) BD wash buffer

90% (v/v) Deionised water + 10% (v/v) 10x stock BD wash solution

(ii) PBS/3 mM EDTA:

112 mg EDTA (AJAX #180) in 100ml PBS, sterile filter using 0.2 µm disposable filter.

(iii) Beta cell culture medium:

RPMI 1640 (Sigma R0883) 200ml

Heat-inactivated fetal calf serum (HIFCS) 20ml

L-Glutamine (Gibco # 25030081 200mM) 2ml (final 2mM)

Penicillin G, MP Biomedicals #02194537, 0.06 mg/ml

Streptomycin, Sigma #S9137, 0.10 mg/ml

Neomycin, Sigma #N6386, 0.10 mg/ml

(iv) PBS/5% HIFCS (FACs Wash buffer):

500ml PBS + 25ml HIFCS

2. Mabs and pAbs:

Rat anti-mouse CD16/CD32 (mouse Fc block), BD Biosciences #553142 (0.5mg/ml)

10E4 (anti-HS) mAb, Amsbio #370255-1(1mg/ml)

mouse anti-mouse collagen type XVIII (Col18A1), Santa Cruz Biotechnol #1837-46 (0.2mg/ml)

Rat anti-mouse CD44 mAb, BD Biosciences #553130 (1mg/ml)

Rat anti-mouse CD138 (SDC1) mAb, BD Biosciences #553712 (0.5mg/ml)

Mouse anti-human heparanase (HPSE) mAb, Insight Biopharmaceuticals #INS-26-1-0000-12 (150mg/ml)

Goat anti-mouse Ig R-PE, Southern Biotech#1010-09 (0.5mg/ml)

Mouse anti-rat kappa PE, Southern Biotech #3090-09 (0.1mg/ml)

Rat anti-mouse Ig FITC, BD Bioscience #553395 (0.5mg/ml)

3. Other reagents/materials:

Accutase, Millipore #SCR005

Cell culture plates: Cellstar #650180(Greiner Bio-one)

Mini tubes, Axygen/Fisher Biotech #MTS-11C

BD Cytotfix/Cytoperm Kit, BD Biosciences #554714

- 1 See Guidelines, "Before starting" .
- 2 Transfer isolated mouse islets to a 15 ml tube and remove excess medium using a Pasteur pipette. Resuspend in ~10–15 ml PBS/3mM EDTA. Centrifuge at 249g.
- 3 Resuspend the islets in PBS/3mM EDTA. Centrifuge at 249g then carefully remove the supernatant.
- 4 Gently resuspend each pellet in pre-thawed Accutase (250 μ l/500 islets) and place tubes in 37°C waterbath for 10 mins (Note: at 4 min and 8 min, gently knock the pellet to resuspend the islets).
- 5 Dissociate the islets by pipetting up and down 10–15 times using a 1ml single channel pipette.
- 6 Add 10ml culture medium to each tube to terminate the Accutase reaction and centrifuge for 5 min at 249g.
- 7 Discard the supernatant, resuspend in beta cell culture medium (500 μ l/500 islets) and determine cell density (using hemocytometer).
- 8 Transfer islet cells to culture plate, $4-8 \times 10^4$ cells /well and adjust the volume in the wells to 200 μ l by adding beta cell culture medium.
- 9 Centrifuge cells at 249g for 3 min at 23°C. Remove supernatant by flicking.
- 10 For intracellular staining, resuspend separate wells of islet cells in 100 μ l BD Cytofix/Cytoperm. Treat for 10 min at room temperature. Add 100 μ l BD wash buffer and spin again at 249g for 3 min at 23°C.
- 11 Flick off the supernatant and wash the cells in 200 μ l BD wash buffer and centrifuge at 249g for 3 min at 23°C.
- 12 Incubate cells for 30 min on ice with:
25 μ l/well of 10E4 anti-HS mAb diluted to 20 μ g/ml with BD wash buffer or
25 μ l/well of anti-Col18 mAb diluted to 4 μ g/ml with BD wash buffer or
25 μ l/well of anti-SDC1 diluted to 20 μ g/ml with BD wash buffer or
25 μ l/well of anti-CD44 mAb diluted to 40 μ g/ml with BD wash buffer or
25 μ l/well of anti-HPSE mAb diluted to 1.5 μ g/ml with BD wash buffer.



Protect from light.

- 13 Wash 2x with BD wash buffer, as for Step 11.
- 14 Incubate cells for 30 min on ice with
25 µl/well of Goat anti-mouse Ig PE (for HS and Col18) diluted to 2.5 or 5 µg/ml with BD wash buffer or
25 µl/well of mouse anti-rat kappa PE (for SDC1 and CD44) diluted to 2µg/ml with BD wash buffer
25 µl/well of rat anti-mouse Ig FITC (for Hpse) diluted to 10µg/ml with BD wash buffer.
Protect from light.
- 15 Wash 2x with BD wash buffer, as for Step 11.
- 16 Resuspend cells in 100µl /well BD wash buffer, transfer cells from each well to an individual mini tube and run samples on flow cytometer.
- 17 For cell surface staining on separate aliquots of cells, apply steps 9, 12-16 (inclusive), with the exception that all washes and antibody dilutions are done in FACs wash buffer.
- 18 Cells with cell surface or intracellular HS, HSPGs or Hpse are collected using a BD LSR Fortessa flow cytometer with BD FACS DIVA software (version 8). Data is analysed using FlowJo software (version 10.0.7, TreeStar Inc.).