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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.

Troubleshooting

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 Cytofix/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 µl/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 µl/500 islets) and determine cell density (using hemocytometer).

8 Transfer islet cells to culture plate, 4-8 × 10^4 cells /well and adjust the volume in the wells to 200 µl 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 100µl BD Cytofix/Cytoperm. Treat for 10 min at room temperature. Add 100µl BD wash buffer and spin again at 249g for 3 min at 23°C.

11 Flick off the supernatant and wash the cells in 200µl BD wash buffer and centrifuge at 249g for 3 min at 23°C.

12 Incubate cells for 30 min on ice with:
25 µl/well of 10E4 anti-HS mAb diluted to 20µg/ml with BD wash buffer or
25 µl/well of anti-Col18 mAb diluted to 4µg/ml with BD wash buffer or
25 µl/well of anti-SDC1 diluted to 20µg/ml with BD wash buffer or
25 µl/well of anti-CD44 mAb diluted to 40µg/ml with BD wash buffer or
25 µl/well of anti-HPSE mAb diluted to 1.5µg/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.).