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Immunohistochemical staining of heparanase (Hpse) in islets of formalin-fixed human pancreas

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Lora Starrs, Debra Brown, Sarah Popp¹, Andrew Ziolkowski, Charmaine Simeonovic¹

¹The John Curtin School of Medical Research, The Australian National University

Charmaine Simeonovic

ABSTRACT

Paraffin sections (4 µm thickness) of formalin-fixed human pancreases were treated with heat/citrate buffer for antigen retrieval. Hpse was detected immunohistochemically using HP130 mouse anti-human Hpse mAb (Insight), with biotinylated anti-mouse IgG (1/250) (PK-2200, Vector Labs) and avidin-biotin-complex (ABC reagent; PK-2200, Vector Labs). Background staining was checked using the corresponding isotype control Ig instead of the primary antibody. 3-amino-9-ethylcarbazole (AEC) was used as the chromogen. Stained sections were imaged using a light microscope with attached camera (Olympus BX41).

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BEFORE START INSTRUCTIONS

Materials:

1. Prepare graded alcohols and xylene for deparaffinizing tissue sections: 2 x xylene (250 ml/slide container), 2 x absolute ethanol (250 ml/slide container), 1 x 90% ethanol (250 ml), 1 x 70% ethanol (250 ml).

1. Prepare acetate buffer components:
(i) 0.1N acetic acid: 290 µl glacial acetic acid in 50 ml deionized water
(ii) 0.1M sodium acetate: 410 mg anhydrous CH₃COONa in 50 ml deionized water.
Prepare 0.1M acetate buffer (pH 5.2) by mixing 10.5 ml 0.1N acetic acid and 39.5 ml 0.1M sodium acetate.

1. Prepare stock solution of 3-amino-9-ethylcarbazole (AEC; chromogen, 8 mg/ml: 40 mg AEC in 5 ml N-N-dimethyl formamide; protect from light and refrigerate at 4°C.
1. Prepare M.O.M. diluent: 200 µl M.O.M. protein concentrate stock solution (M.O.M. Immunodetection kit, PK-2200, Vector) in 2.5 ml phosphate-buffered saline (PBS) for use either as a blocking step to minimize non-specific Ig binding or for diluting antibodies.

1. Mabs and pAbs:
HP130 mouse anti-human heparanase mAb, Insight Biopharmaceuticals #INS-26-1-0000-21 (alternative: HPA1 rabbit anti-human heparanase, Insight Biopharmaceuticals #INS-26-2-0000)
Biotinylated anti-mouse IgG, M.O.M immunodetection kit, Vector Labs # PK-2200 (alternative: HRP-swine anti-rabbit Ig, Dako #PO217)
Mouse IgG1_K, eBioscience # 14-4714-85 (alternative Rabbit IgG, Southern Biotech, #0111-01)

1. Other reagents:
Hydrogen peroxide (30% w/w), Chem-Supply Pty Ltd (Australia) #HA154-500M
Methanol, Merck # CAS-No. 67-56-1
3-Amino-9-ethylcarbazole (AEC), Sigma-Aldrich #A5754
Animal free blocker, Vector Labs #SP-5030
Stock protein concentrate, M.O.M immunodetection kit, Vector Labs # PK-2200
Avidin-biotin complex (ABC), M.O.M immunodetection kit, Vector Labs # PK-2200
N-N-dimethyl formamide, Sigma #D158550
Glycergel mounting medium, Dako #C0563

- 1 See Guidelines, "Before starting"
- 2 Deparaffinize slides in each xylene for 1 min. rehydrate slides in graded alcohols beginning in absolute ethanol (10 dips)/ container of absolute ethanol), followed by 90% ethanol (10 dips) and 70% ethanol (10 dips). Wash well in running tap water for 5 min.
- 3 Wipe around sections with a tissue, encircle the sections using a diamond pencil and place in a slide container of tap water (250 ml).
- 4 Block endogenous peroxidase activity by incubating sections in 3% hydrogen peroxide in methanol (25 ml 30% H₂O₂ + 225 ml methanol).
- 5 Wash 2 x 2 min in 250 ml phosphate-buffered saline (PBS) followed by wash in running tap water for 5 min.
- 6 Prepare citrate buffer, pH 6 for antigen retrieval. Dissolve 1.05 g Citric acid in 500 ml deionized water and pH using 2 - 10 M NaOH.
- 7 Transfer slides to 250ml citrate buffer and heat in microwave (1600 watt) for 2 min on High power followed by 2 x 6 min on Low power. Allow the slides to cool on the bench for 30 min. Wash slides in 250 ml PBS, 3 x 10 min.
- 8 Wipe around sections using tissue. To block non-specific binding of Ig, apply M.O.M. diluent to tissue sections and incubate for 5 min at room temperature.
- 9 Tip off excess block in Step 7, wipe around sections using tissue and incubate with 340 µg/ml HP130 anti-Hpse mAb (or 350 µg/ml mouse IgG_{1K} as isotype control; diluted in M.O.M. diluent),

125-150 µl/section at room temperature for 30 min.

(Alternative: 228 µg/ml HPA1 rabbit anti-human heparanase IgG or 227 µg/ml rabbit IgG as isotype control).

- 10** Wash off primary antibody with PBS and transfer slides to slide container with 250 ml PBS. Wash 2 x 2min.
- 11** Wipe around sections using tissue and incubate with 1/250 diluted secondary biotinylated-anti-mouse IgG (M.O.M. immunodetection kit), 150 µl/section, for 10 min at room temperature.
(Alternative: 37 µg/ml HRP-swine anti-rabbit Ig)
- 12** Wash off secondary antibody with PBS and transfer to slide container with 250 ml PBS. Wash slides 2 x 2min.
- 13** Wipe around sections using tissue and cover with Vectastain ABC reagent (M.O.M immunodetection kit), for 5 min at room temperature.
(alternative: delete Step 13 if HPA1 anti-heparanase pAb is used)
- 14** Wash off ABC reagent with PBS and transfer to slide container with 250 ml PBS. Wash slides 3x in 10 min.
(alternative: delete Step 14 if HPA1 anti-heparanase pAb is used)
- 15** Prepare AEC working solution: 4.75 ml acetate buffer (see Guidelines), 0.25ml AEC stock solution and 25 µml 3% H₂O₂. Filter using a disposable 0.2 µm filter. Use within 2 hours of preparation, refrigerate for short-term storage. Protect from light.
- 16** Wipe around sections using tissue and cover the sections with AEC solution for 30 min at room temperature.
- 17** Wash off AEC solution with deionized water and transfer slides to slide container with 250 ml deionized water. Wash 3x in 10min.

- 18** Lightly counterstain with Mayer's hematoxylin, wash in deionized water (2 x) and briefly dip in ammonium water (100 µml ammonia in 250 ml deionized water), 2 x 2 sec. Wash in deionized water (2 x in 250 ml) and coverslip using glycerol mounting medium.
- 19** Photograph sections using a light microscope with camera attachment.