Immunohistochemistry analysis

CURRENT STATUS: POSTED

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DOI:
10.1038/nprot.2006.199

SUBJECT AREAS
Biological techniques

KEYWORDS
trophoblast, chemokine receptors, natural cytotoxicity receptors

Introduction

Immunohistochemistry protocols

Reagents

Primary monoclonal mouse anti human antibodies (all obtained from R&D Systems, Inc. MN. USA): anti-human CXCR3 antibody (IgG1, clone49801, staining concentration of 10 micrograms/ml), anti-human CXCR1 mAb (IgG2A, clone 42705, staining concentration of 10 micrograms/ml), anti-human CXCR4 (IgG2A, clone 44708, staining concentration 10 micrograms/ml).

The mouse anti human mAb for HLA-G was previously described and kindly provided by Dr. Mike McMaster (mouse IgM, staining concentration of 10 micrograms/ml) [2].

Procedure

1) Perform immunohistochemistry for chemokine receptors CXCR1, CXCR3 and CXCR4 on first trimester human placental paraffin-embedded sections as previously described [1], with microwave antigen retrieval using sodium citrate buffer (Zymed Laboratory Inc, San- Francisco, CA, USA).

2) Stain with the same concentration of isotype match controls for each chemokine receptor on the same serial sections as controls.

3) Perform immunohistochemistry on decidual sections with NCR-Fc fusion proteins (prepared as previously described 3) by microwave heating the formalin-fixed, paraffin embedded tissue sections in citrate buffer in order to retrieve antigens.

4) Then stain sections with the different NCR-Fc fusion proteins or control-Fc (8 µg/ml, final concentration) followed by biotinylated-goat-anti-human-Fc (Jackson ImmunoResearch, West Grove, PA).

5) Detect using the avidin-biotin peroxidase complex method with a Vectastain kit (Vector Laboratories, Burlingame, CA).

References

1. Hanna, J. *et al.* CXCL12 expression by invasive trophoblasts induces the specific migration of CD16- human natural killer cells. *Blood* **102**, 1569-77 (2003).
2. McMaster, M.T. *et al.* Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J Immunol* **154**, 3771-8 (1995).

3. Arnon, T.I. *et al.* Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus. *Nat Immunol* **6**, 515-23 (2005).

Decidual NK cells regulate key developmental processes at the human fetal-maternal interface

by Hanna, J. et al.

Nature Medicine (09 June, 2006)