Immunohistochemistry analysis

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Method Article

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Abstract

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).