Rtl1 and CD31 double-immunohistochemistry

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Introduction

This is a protocol to observe Rtl1 protein localization in placenta. There are three kinds of trophoblast cells and endothelial cells of fetal capillary in placental labyrinth zone. CD31 is a marker protein of endothelial cells. Therefore, nuclei out of green signal of CD31 are of the trophoblast cells.

Procedure

1. Embed fresh placenta in OCT compound immediately after picking up from uterus.
2. Freeze the compound in cooled 2-methyl butane in a liquid nitrogen bath.
3. Cut into 5-µm sections on a cryostat and dry them at room temperature.
4. Fix the sections in 4% paraformaldehyde for 10 min.
5. Wash the slides in 0.01M PBS for 5 min three times.
6. Put the slides in 0.3% H_2O_2/Methanol for 15 min.
7. Wash the slides in 0.01M PBS for 10 min three times.
8. Apply a drop of normal goat serum to each specimen and incubate for 10 min in moistening box at room temperature.
9. Discard the serum, apply a drop of 1st antibody mixture to each specimen and incubate for 1 hr at room temperature in the moistening box. 1st antibody mixture for each specimen: anti-Peg11 antibody 1 µl + anti-CD31 (Pecam-1) (BD Pharmingen) 2 µl + 1% BSA 97 µl
10. Wash the slides in 0.01M PBS for 10 min three times.
11. Apply a drop of normal goat serum to each specimen and incubate for 1 min in moistening box at room temperature.
12. Discard the serum, apply a drop of 2nd antibody mixture to each specimen and incubate for 1 hr at room temperature in dark moistening box. 2nd antibody mixture for each three specimens: Alexa Fluor 488 goat anti-rat IgG (Invitrogen) 1.5 µl + Cy3-conjugated AffiniPure Goat anti-rabbit IgG (Jackson ImmunoResearch) 1 µl + Hoechst33342 (Invitrogen) (1/100 diluted by DDW) 1µl + 1% BSA 300 µl

13. Wash the slides in 0.01M PBS for 10 min three times.

14. Apply a drop of 50% glycerol to each specimen, cover it with coverslip and completely seal the edges of coverslip with nail polish.

15. Observe under fluorescence microscope. (Excitation, Emission)

Alexa 488 (495, 520)

Cy3 (512/552, 570)

Hoechst33342 (360, 450)

Role of retrotransposon-derived imprinted gene, Rtl1, in the feto-maternal interface of mouse placenta

by Yoichi Sekita, Hirotaka Wagatsuma, Kenji Nakamura, +10
Nature Genetics (24 October, 2007)