

Placental CD24 and its association with Siglec-10 in the decidua during the first trimester of pregnancy

Marei Sammar¹, Monika Siwetz², Niko P. Bretz⁴, Hamutal Meiri³, Peter Altevogt⁴, Berthold Huppertz²

¹Prof. Ephraim Katzir Department of Biotechnology Engineering, ORT Braude College, P.O. Box 78, Karmiel 21982, Israel, sammar@braude.ac.il

²Institute of Cell Biology, Histology and Embryology, Medical University of Graz, 8010 Graz, Austria. ³Hylabs, Rehovot and TeleMarpe, Tel Aviv, Israel. ⁴Tumorimmunology, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany



Medical University of Graz

Introduction

The fetal-maternal interface acquires immune-tolerance between the fetus and the mother. The process involves cell-cell interaction regulated by several glycoproteins to dampen the immune response. Here we investigated two candidates for a new immuno-suppression regulation of this process: 1) The signal transducer CD24, a sialo-glycoprotein expressed at the surface of some hematopoietic cells, differentiating neuroblasts and diverse tumor cells, serving as cell adhesion molecule for P-selectin and Siglecs. And 2) Sialic acid-binding immunoglobulin-type lectins (Siglecs).

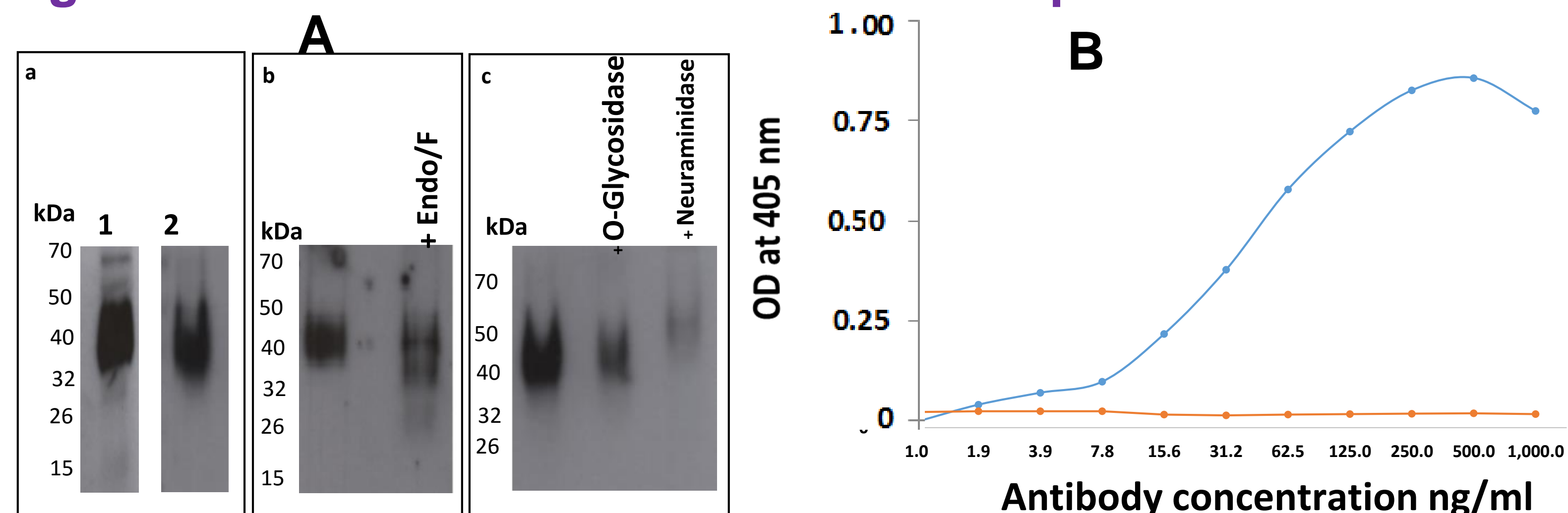
Methods:

- CD24 was affinity purified from term placental and characterized by separation on SDS-PAGE followed by Western blot and ELISA analysis.
- CD24 associated carbohydrate residues were studied by glycosidases treatment and by its reactivity with lectin.
- The interaction of recombinant Siglecs with placental CD24 was measured by ELISA. CD24 expression in first trimester placental and decidual tissues was studied by immunohistochemistry and immunofluorescence.

Aim of the study: To study the role of CD24 in the placenta by identifying where it is expressed and with which proteins it interacts within the placenta during the first trimester of pregnancy.

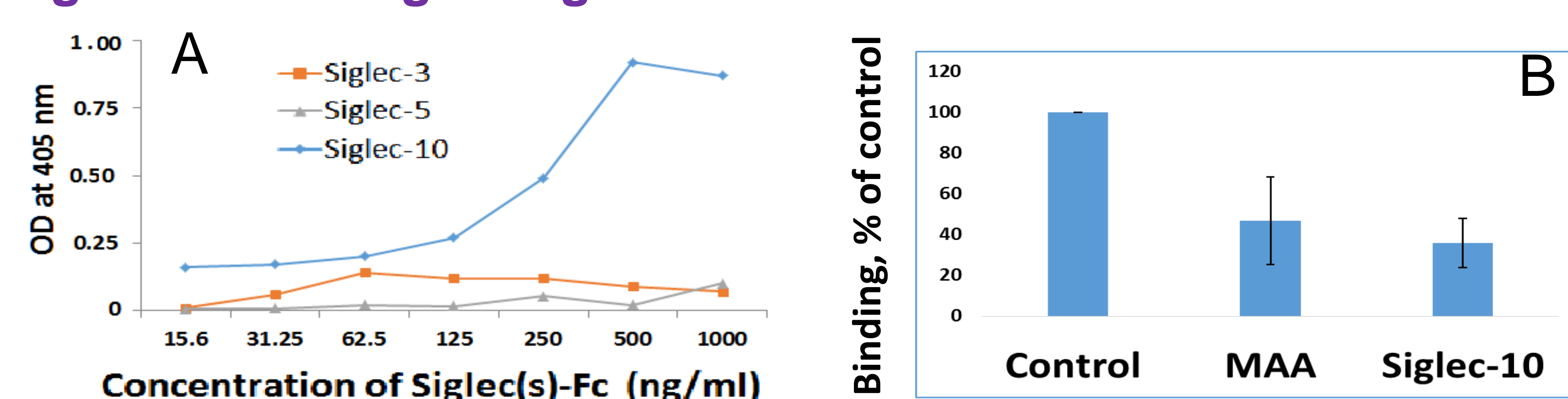
RESULTS

Figure 1: Biochemical characterization of placental CD24



Western blot (A) and ELISA immunoassays (B). Placental CD24 migrates as a diffused band (MW=30-70kDa, a 1+2) which is sensitive to several glycosidases (b) and (c). Antibodies from clone SWA11 against CD24 recognize CD24 in a dose dependent manner (blue in B). The isotype control antibody didn't react with CD24 (red in B).

Figure 2: Binding of Siglec-Fc Proteins to Placental CD24



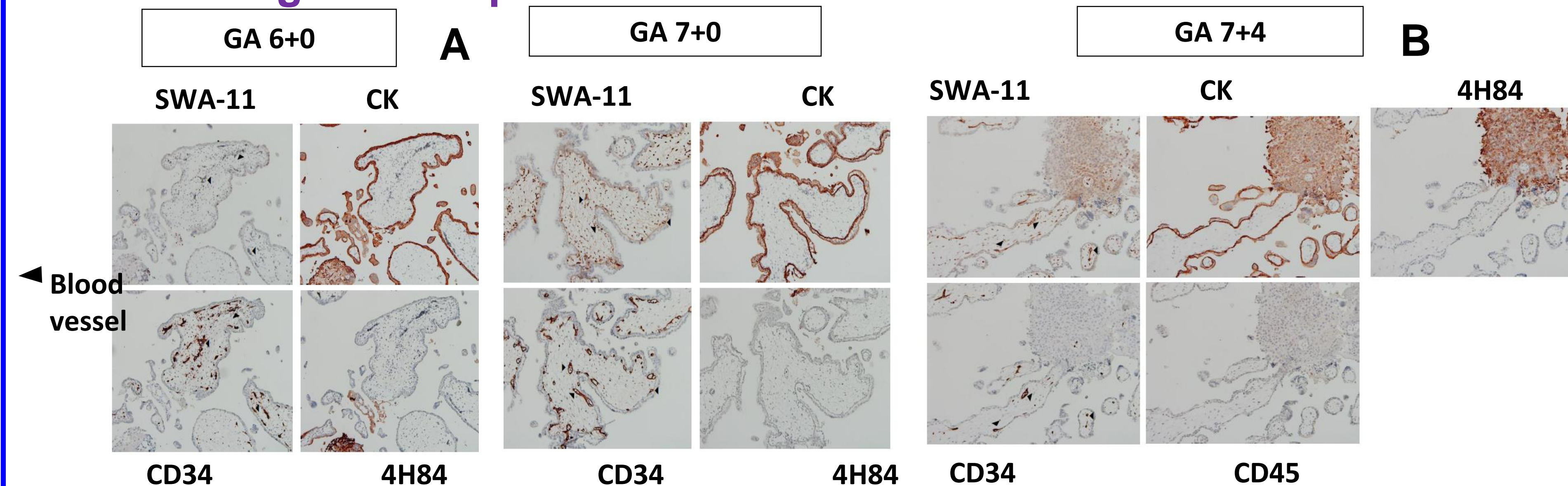
Recombinant human Siglec-10 but not Siglec-3 or Siglec-5 shows strong dose dependent binding to placental CD24 in ELISA (A). Treatment of CD24 by neuraminidase (B) significantly reduced the binding of Siglec-10 (65%) and of MAA specific lectin (45%) to sialic acid, which was used as a positive control.

Conclusions

- Antibodies to anti-CD24 (clone SWA-11) interact with placental CD24 under native and denaturated conditions and are suitable for immunohistochemistry and immunofluorescence.
- Placental CD24 is a heavily glycosylated protein specifically interacting with Siglec 10 via its sialic acid glycan moieties.
- CD24 is intensely expressed in villous cytotrophoblast, placental endothelial cells and stroma cells and maternal uterine glands.
- CD24 co-localizes with Siglec 10 in endometrial glands.
- The results indicate that CD24-Siglec 10 interaction may be involved in the immune tolerance at the fetal-maternal interface of early stage placentation. More studies are needed to examine the significance of this interaction.

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Figure 3: Expression of CD24 in the first trimester on Villi



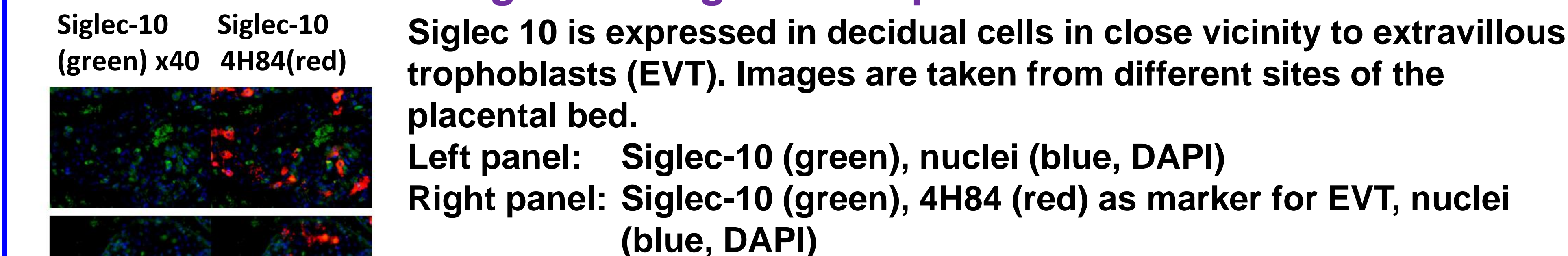
A - CD24 is expressed in villous cytotrophoblast but absent from the syncytiotrophoblast. CD24 is also expressed in endothelial cells and specific stroma cells.
B - CD24 is expressed in extravillous trophoblasts (EVT).
SWA-11 is a CD24 marker, CK7-cytokeratin 7 is a trophoblast marker, CD34 labels endothelial cells, 4H84 labels extravillous trophoblast (EVT).

Figure 4 : CD24 expression in the Decidua



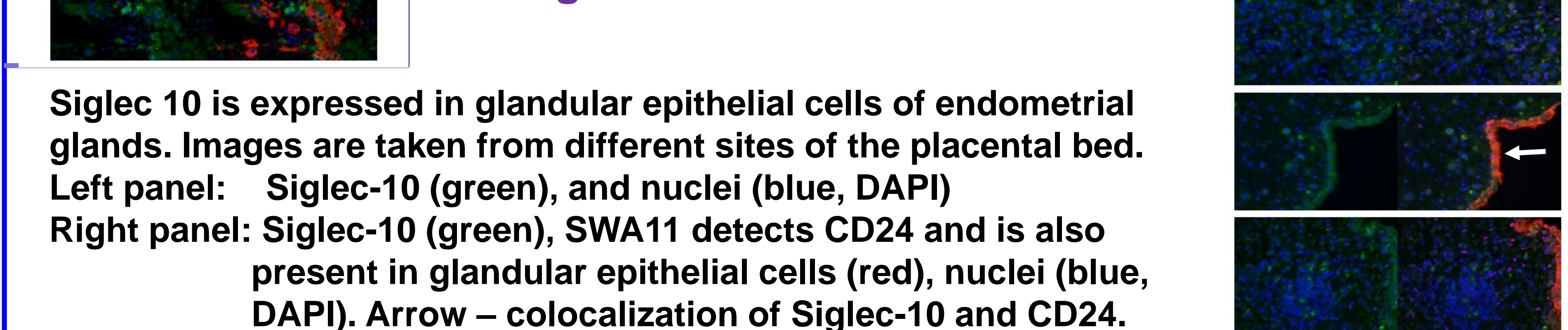
CD24 is expressed in maternal uterine glands (asterisks in 1,2) but absent from maternal decidua vessels (asterisks in 3,4) and stroma cells.
SWA-11 is a CD24 marker, CK7-cytokeratin 7 is a trophoblast marker, CD34 labels endothelial cells, 4H84 labels extravillous trophoblast.

Figure 5 : Siglec 10 Expression in EVT



Siglec 10 is expressed in decidual cells in close vicinity to extravillous trophoblasts (EVT). Images are taken from different sites of the placental bed.
Left panel: Siglec-10 (green), nuclei (blue, DAPI)
Right panel: Siglec-10 (green), 4H84 (red) as marker for EVT, nuclei (blue, DAPI)

Figure 6 : Co-localization of CD24 and Siglec 10 in Endometrial Gland



Siglec 10 is expressed in glandular epithelial cells of endometrial glands. Images are taken from different sites of the placental bed.
Left panel: Siglec-10 (green), and nuclei (blue, DAPI)
Right panel: Siglec-10 (green), SWA11 detects CD24 and is also present in glandular epithelial cells (red), nuclei (blue, DAPI). Arrow - colocalization of Siglec-10 and CD24.