Vascular smooth muscle cell fate in spiral artery remodelling in early human pregnancy

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Introduction

- Human uterine spiral artery remodelling is a key maternal adaptation to pregnancy
- Failure of this process is associated with preeclampsia, fetal growth restriction, late miscarriage and preterm delivery
- Spiral arteries are composed of vascular smooth muscle cells (VSMCs) which make the wall elastic, extracellular matrix and endothelial cells
- During remodelling, the VSMCs and extracellular matrix are replaced by rigid fibrinoid tissue and extravillous trophoblast cells (EVT)
- This results in dilation of the arteries as their role is to provide the placenta and fetus with necessary oxygen and nutrients
- Dilated vessels can deliver more blood at a steady flow to the fetoplacental unit
- The fate of VSMCs after they are replaced is not fully understood
- Preliminary data suggests that these cells migrate away from the artery wall into the decidual stroma, undergo apoptosis and are then phagocytosed by macrophages

Aims

- To estimate the association of migrated vascular smooth muscle cells with macrophages using Immunostaining of placental bed biopsies

Double Immunohistochemistry

Placental bed biopsies and decidua from early pregnancy were assessed immunohistochemically for the following antibodies:

- **Hcaldesmon** for vascular smooth muscle cells (1/100 dilution)
- **CD14** for macrophages (1/20)
- **Lamin** for an apoptotic marker (1/20)

Double labelling immunohistochemistry was performed on placental bed biopsy tissue to quantify the VSMC migration. The quantification was performed by estimating the following:

1. Number of migrated VSMCs, number of migrated VSMCs associated with macrophages to estimate the % of migrated VSMCs associated with macrophages
2. Number of migrated VSMCs, number of migrated VSMCs without Lamin (a marker lost during apoptosis) to estimate the % of migrated VSMCs that are apoptotic.

There was a lot of variation in the percentage of migrated VSMCs associated with macrophages, ranging from 11% to 100%. The average of migrated VSMCs associated with macrophages was 42% (Figure 1 and 2)

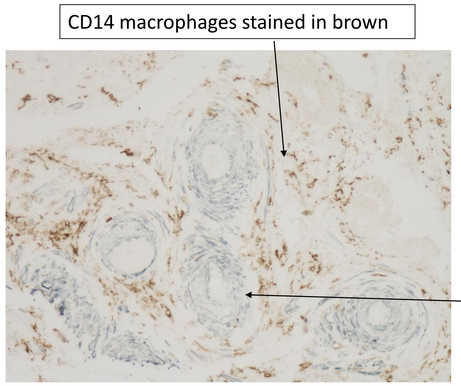

Figure 1: Double labelling immunohistochemistry (CD14/Hcal antibodies) of placental bed biopsy tissue. From this picture we can estimate the % of migrated VSMCs associated with macrophages

Figure 2: Table showing the % of migrated VSMCs associated with macrophages. The results were obtained from double immunohistichemistry (CD14/Hcal)

CD14/HCal				
Sample number		Number of migrated VSMCs	Number of migrated VSMCs associated with macrophages	% of migrated VSMCs associated with macrophages
1	Vessel 1	6	3	50.0%
	Vessel 2	11	3	27.3%
	Vessel 3	1	1	100%
	Vessel 4	6	4	66.7%
2	Vessel 1	20	11	55.0%
3	Vessel 1	19	3	15.8%
4	Vessel 1	9	1	11.1%
	Vessel 2	6	1	16.7%

Triple Immunohistochemistry

- While we could show that migrated VSMCs were apoptotic and associated with macrophages we could not tell from the double labelling experiments whether the macrophages were specifically associated with apoptotic VSMCs
- Therefore, triple labelling was performed for nuclear Lamin, CD14 and H-cal. We focused on myometrium and nonvascular smooth muscle cells, which can behave in a similar manner to vascular ones.
- Representative photomicrographs of immunostatining in Figure 3 and 4 show the follwing:

1. Viable smooth muscle cell (SMC)
2. Apoptotic SMC
3. CD14 macrophage
4. Macrophage associated with apoptotic SMC

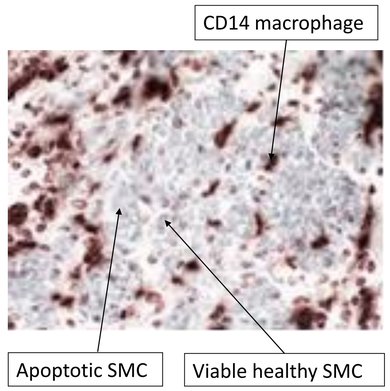

Figure 3: Triple immunohistochemistry performed on smooth muscle cells section in myometrium. In the picture we can distinguish a viable SMC, an apoptotic SMC and CD14 macrophage

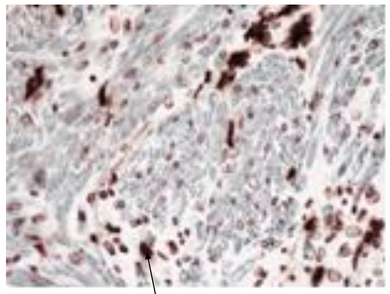

Figure 4: Triple immunohistochemistry performed on smooth muscle cells section in myometrium. In the picture we can distinguish an apoptotic SMC associated with CD14 macrophage

Conclusions and further studies

- In general, vascular smooth muscle cells were observed migrating into the decidual stroma during the spiral artery remodelling
- Around 42% of the VSMCs associated with macrophages after migration from the vessel wall
- Some of the migrated VSMCs which associated with CD14 macrophages were also found to be apoptotic
- Triple labelling of placental bed biopsy tissue with different antibodies and stains should be conducted to obtain a more distinct image of vascular smooth muscle cell fate and quantify the level of apoptotic VSMCs associated with macrophages

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