

The intermediate filament Nestin is not a specific marker for proliferating endothelium – its expression *in situ* and *in vitro*

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INTRODUCTION: For several Tissue Engineering strategies the determination of proliferating endothelial cells is of special interest. In this context, the intermediate filament nestin is expressed by endothelial cells (ECs) of regenerative tissues and malignant tumours. It is widely believed that nestin is a marker for proliferative endothelium only. In the present study, we documented the *in situ* expression of nestin in the endothelium of the human cardio-vascular tree as well as in human haemangiomata and human lymphangiomata. Furthermore, we investigated the *in vitro* expression of nestin by cultured ECs of different origin.

METHODS: Human aorta (n=5), vena cava (n=5), arteria et vena renalis (n=5), capillaries in liver and lung (n=5), haemangiomata (n=5) and lymphangiomata (n=5) were analyzed immunohistochemically using the peroxidase method. We used the panendothelial marker CD31 and the lymphatic marker D2-40. Nestin was detected with a monoclonal antibody. Proliferating cells were visualized using antibodies against Ki67. For *in vitro* analyses, we cultured human umbilical vein ECs (HUVECs), human pulmonary microvascular ECs (HPMECs), and an immortalized HPMEC cell line (HPMEC-ST1). In subconfluent and confluent populations the cells were analyzed by immunofluorescence for expression of CD31 and nestin.

RESULTS: In all specimens of aorta, vena cava, arteria et vena renalis, the capillaries of liver and lung ECs were positive for nestin. *In situ* all EC reacted negatively with antibodies against Ki67. All haemangiomata were strongly positive for nestin and negative for D2-40. In contrast, the endothelium of the lymphangiomata showed a homogeneous expression of D2-40 and showed no reaction with monoclonal anti-nestin antibodies. *In vitro*, HUVEC, HPMEC and HPMEC-ST.1 showed no expression of CD31 in subconfluent cultures. When confluent, strong CD31 expression could be demonstrated at the intercellular contacts. In the subconfluent as well as in the confluent status, cultured ECs expressed nestin.

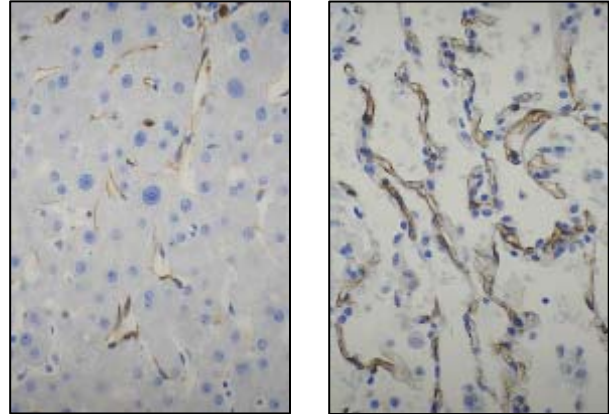


Figure 1, 2: Nestin expression in human liver (left) and lung capillaries (right).

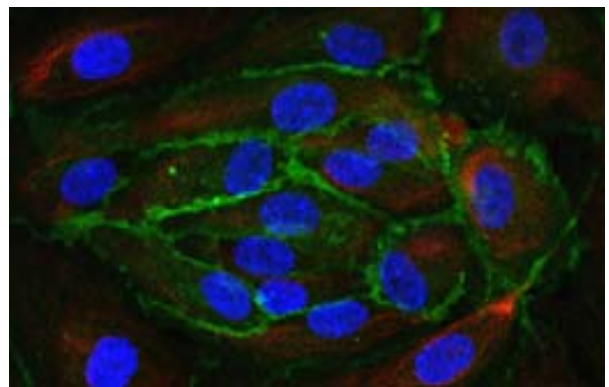


Figure 3: Nestin expression (red) in a confluent HUVEC monolayer with CD31 (green) and Hoechst (blue).

DISCUSSION & CONCLUSIONS: Nestin is expressed in endothelial cells of arteries, veins and capillaries within the cardio-vascular tree as well as in confluent EC cultures of different tissue origins. The differential expression of nestin in haemangiomata and lymphangiomata indicates the specificity of nestin for the endothelium of blood vessels. Our findings contradict the current view, which defines nestin as a marker for proliferative endothelium. In conclusion, nestin is not a specific marker for proliferating endothelial cells. Therefore, it appears that widely held beliefs about the functional role of nestin in ECs have to be re-evaluated and re-defined in further studies.