6. Summary

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Immunhistochemical detection of the VEGF-receptor *KDR* in histologic sections of monkey tissues

The blood-vessel system plays a pivotal role within the organism. It provides nutrients for the tissues, removes metabolism products, takes care of the gas exchange and various other functions of the body.
The formation of new blood vessels is in certain situations, like wound-healing, essential and physiological. Given these conditions, angiogenesis is highly regulated. Unfortunately this process of new blood vessel formation can get out of control. Angiogenesis is increased in many diseases (i.e. neoplasia).
By discovering VEGF, one important regulator of angiogenesis, vasculogenesis, proliferation of endothelial cells and permeability of blood vessels has been found. Its activity is mediated through specific receptors like KDR. KDR was discovered in 1991 and has been object of many investigations ever since. Despite this fact many questions still remain open.

The aim of this study was to localize the KDR in normal tissues of monkeys for the first time immunohistochemically and by doing so to gain a better understanding of VEGF and his part in healthy organism. In addition the selective expression of KDR on endothelium was to verify. Therefor the method for the immunhistochemical detection of KDR with a monoclonal antibody developed by MENRAD et al. (1997) should be transmitted to the tissues of monkeys.

Method: To demonstrate the KDR immunohistochemically, cryosections were incubated with the monoclonal antibody 2-10-1 according to the two-step-method.
To reconsider the EC-specifity of KDR, the EC-receptor CD31 was detected immunhistochemically.

Results: The VEGF-receptor KDR was identified in several normal tissues of monkeys. Its presence was found on endothelial cells as well as in other non-endothelial tissues.

The results of the immunhistochemical detection of the VEGF-receptor *KDR* can be classified into three groups:

1. Histologic structures, which are staining in correspondence to the CD31-detection:

In all investigated organs, the KDR was expressed in the **endothelial cells from blood vessels**. In the skin, the brain and the thyreoid glands, they were the only KDR positive structures.

2. Cells, which express the CD31-receptor but not the KDR:
In the liver, EC from blood vessels of the Glisson's sling did not show any KDR-expression.

3. Cells, which express the KDR but not the CD31-receptor:
In the **kidney**, cells of the tubules of the main parts did participate in the KDR-expression.
In the lymphatic organs like **lymph node**, **spleen** and **thymus**, lymphocytes has been discovered as carriers of the KDR. The mesothelium of spleen and isolated fibroblasts in the thymus showed a positive immunohistologically staining of KDR as well.
The capillaries of **liver** sinus with discontinuous endothelium demonstrated a strong KDR-expression.
In the **pancreas**, cells showed a KDR-expression, not only in the endocrine but also in the exocrine part.
In the **trachea**, epithelium and chondrocytes showed a KDR-expression. Again the fibroblastes were detected as KDR positive.
The muscle cells of the **heart** as well as the isolated fibroblasts/-cytes were identified as KDR-expressed cells.
In the organs of the **gastrointestinal tract**, smooth muscle cells, the mesothelium and epithelial cells of glands presented the KDR. Additionally, isolated fibroblasts and lymphocytes in the lymph nodes showed a positive staining.

Conclusions: With respect to these findings an immunohistochemical detection of the VEGF-receptor *KDR* with the monoclonal antibody 2-10-1 is possible in cryosections of monkey tissues.
According to these investigations, the KDR-expressing cells show a wide distribution in normal monkey tissues. Therefore it is an educated guess, that the KDR, who was for a long time considered the endothelial specific receptor of VEGF, is not only expressed by these cell types.
The results in hand confirm, that there exists a reciprocal action between the occurrence of KDR on endothelial cells and fenestration of capillaries. In addition, the KDR was detected on non-endothelial cells, i.e. epithelium of glands, lymphocytes and smooth muscle cells. These findings not only confirm the results of other investigations, but also lead to the conclusion, that VEGF possesses non-endothelial target-cells, too. This thesis is supported by the results of the chondrocytes, the muscle cells of the heart and the fibro-

blasts/-cytes, which were identified as KDR-expressing structures in the study in hand for the first time.

The functions of VEGF in other cell types than EC are not completly clear yet.VEGF plays a role in immunology in addition of his function as a angiogenesis-stimulator. The results in hand can be serve as a basis for further investigations to clarify other functions of VEGF.