Summary

The development of antibodies against cell specific surface markers allows the selective application of therapeutic effectors that can be used for antibody targeted delivery after antibody coupling. Apart from the choice of the effector, the therapeutic effect depends on the specificity of the target structure. Therefore, new and specific target molecules are of outstanding medical interest. Phage display is an effective and established method for the selection of antibodies from scFv phage libraries against proteins. The search for antibodies against unknown antigens such as cell or tissue specific markers requires selection strategies on complex antigen sources. For the development of anti-angiogenic approaches e.g. appropriate target structures on endothelial cells must be found.

In this thesis a phage display method for the isolation of highly specific antibodies on primary endothelial cells was established. Using this surface selection, scFv phages with high endothel cell specificity could be isolated. Cell specificity of these phage antibodies was confirmed by ELISA experiments and immunohistochemical staining of blood vessels on slides from healthy and tumor tissue. The application of antibodies in immunotoxins or in gene therapy depends on a targeted transport into the cell. Until today, successful antibody phage display strategies for the selection of internalizing scFv-phages have mostly been described on cell lines which overexpress a receptor molecule such as the EGF receptor on transfected cells or certain tumor cell lines. An extension of the described surface selection on primary endothelial cells allowed the isolation of internalizing scFv-phage. The comparison of two different internalizing selection strategies on endothelial cells showed that a multivalent presentation of antibody fragments is necessary to isolate internalizing scFv-phage. With a modified scFv-phage library that allowed a multivalent presentation of scFv-fragments on the phage surface, three different scFv phages were isolated. Internalization of these three phage antibodies could be shown by means of immunofluorescence microscopy experiments. The established protocols of this work can be used as starting point to perform and improve cell selections against surface specific or internalizing antigens under more controlled conditions in the future. Furthermore, this methodology will supplement existing antigen identification techniques. The accessible antigen structures could possibly be used as new therapeutic or diagnostic markers.