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An *in vitro* model of angiogenesis of endothelial cells from the murine myocardium and from the human neonatal foreskin and transfection of endothelial cells with different plasmid constructs

Angiogenesis, the formation of new blood vessels by endothelial cells, plays an important role during prenatal growth as well as postnataly in diseases such as tumor growth. Targeting endothelial cells by gene transfer to inhibit angiogenesis offers an attractive anticancer approach. A selective expression of the transgene by endothelial cells can be achieved by use of endothelial-specific gene regulatory elements. The present work pursued two main aims, namely the establishment and characterization of *in vitro*-models of angiogenesis of murine and human microvascular endothelial cells as well as the characterization of different plasmid constructs on efficiency in these models. Furthermore morphological alterations of transfected endothelial cells should be examined on light and electron microscopic basis in order to make statements about the uptake of the complexes by the cells and their way to the nucleus as well as to appraise possible morphological damages of the cells caused by transfection.

The angiogenic cascade of endothelial cells, isolated from the human neonatal foreskin and from the myocardium of two weeks old mice, was induced by pro-angiogenic factors. The beginning of the angiogenic cascade was initiated morphologically by a complete as well as partial confluence of the endothelial cells (*stage 1*). A linear and circular side by side arrangement of cells (*stage 2*) and the formation of capillary-like structures with an internal lumen (*stage 3* and *4*) could be observed. The endothelial cells produced a basement membrane-like material, which was found intercellularly as well as in intracellular vacuoles and in the lumen of capillary-like structures. In lumen formation vacuolization as well as apoptosis were involved. While the invested murine endothelial cells formed capillary-like structures planar to the culture dish surface (two-dimensional *in vitro*-model of angiogenesis), a realistic three-dimensional *in vitro*-model of angiogenesis could be established with the human endothelial cells with strong similarity with angiogenesis *in vivo*. Collagen IV, a basement membrane component, was identified by immunolabeling. An especial result of this

work observed in the murine cell culture model was the cyclic course of *in vitro*-angiogenesis with a „latency time“ between. After detachment of the capillary-like structures of the culture dish a reappearance of these structures could be observed after approximately 2 months cultivation of the remained cells. Interesting was, that the capillary-like structures in the second cycle were formed by endothelial cells, which were uninvolved at the formation of these structures before. The results indicate that a differentiation of the endothelial cells to an angiogenic phenotype took place during the „latency time“.

Endothelial cells at different stages of the angiogenic cascade (*stage 0* versus *3* respectively *4*) were transfected by polyfection with activated dendrimers. Transfection of proliferative endothelial cells (*stage 0*) led to expression of the reporter gene (luciferase) with all vectors invested pJWM115 (CMV-luc), pCK5 (Ets-1l-luc), pPS12 (Ets-1k-luc), pPS6 (E-sel-luc). This indicates the expression of the adhesion molecule E-selectin as well as of the transcription factor Ets-1 by proliferative murine and human endothelial cells. Highest transfection efficiencies were found by using the pJWM115-, pCK5- and the pPS12-vector. The results of transfection of murine and human endothelial cells forming capillary-like structures (*stage 3* resp. *4*) reflected the angiogenic situation in both *in vitro*-models. No reporter gene expression, independently from the vector used, could be detected after transfection of murine endothelial cells at *stage 3*. However, transfection of human endothelial cells at *stage 4* led to an expression, marginal higher than control expression. An important difference between the individual experiments in the human cell culture model could be determined on that occasion. Human endothelial cells that were transfected at beginning of *stage 4*, showed a higher expression of the reporter gene than cells that were transfected at later times of *stage 4*. This is to be explained by the different proliferative rate of endothelial cells at this stage of the angiogenic cascade. These results show a cell cycle dependence of the gene transfer system used in this work. An efficient gene transfer could be observed in proliferative endothelial cells only as result of a raised uptake of complexes as well as a more efficient entry of complexes resp. plasmid DNA into the nucleus. Ultrastructural examination of transfected cells let suspect that the complexes uptaken by endocytosis escape from the endosomes before fusion with primary lysosomes since free DNA-dendrimer-complexes at the nuclear membrane could be detected before appearance of multivesicular bodies (late endosomes) in human endothelial cells. In the murine endothelial cells the intracellular degradation of the complexes seemed to take place more slowly in comparison to human cells which could at least partially explain the broadly higher efficiency of gene transfer in murine cells.
In another experiment the increase of the E-sel promoter activity (pPS6) by two endothelial-specific enhancers 5xebs (pPO18) and flk-1 (pPO14) as well as by the HLA-intron (pPO12) was examined. In contrast to the HLA-intron, which led to a multiple increase of the activity of

the E-sel promoter, promoter activity could not be raised by the endothelial-specific enhancers. This indicates that the corresponding transcription factors like for example Ets-1 are not expressed in the examined cell culture models in sufficient quantity. Gene expression analyses or immunohistochemical examinations of the endothelial cells at different stages of the angiogenic cascade *in vitro* could give more information.

In view of a reduction of animal experiments through substitute and supplement methods, a special meaning comes up to the established *in vitro*-models of angiogenesis. In addition to the animal protection aspect, they represent cost-effective, sensitive, simple experimental systems for continuing examinations. While the murine cell culture model is suitable for gene transfer studies since murine endothelial cells can efficiently be transfected, an experimental system is available with the realistic three-dimensional *in vitro*-model of angiogenesis of human endothelial cells which is suitable for examinations of *in vitro*-angiogenesis. On the basis of the lacking use of a three-dimensional extracellular matrix and the connected reduction of not resp. difficult to standardize factors this model is suitable for examinations of cell adhesion molecules, cell matrix interactions or also for identification of specific inhibitors of lumen formation. The established endothelial cell cultures already are used for similar experiments in other laboratories at present.