7 SUMMARY

In vitro - investigations of the influence of altered gene expression of the TGF-β-binding protein LTBP-4 on the expression pattern of the tumorsuppressive gene p21 and the protooncogene c-myc in HEK293T cells

The expression of genes regulating cell growth and proliferation, such as TGF-β1, p21, and c-myc, can be altered or modified in the process of carcinogenesis in epithelial tumors and therefore results in uncontrolled cell cycle progression.

TGF-β1 (transforming growth factor beta 1) in association to its binding protein sLTBP-4 (»short form« of latent TGF-β binding protein 4) which is an important modulator of TGF-β1-bioavailability regulates different functions in epithelial cells. Its antiproliferative and therefore tumorsuppressive effects represent a major cellular function.

A reduced availability of TGF-β1 results in reduced expression of tumorsuppressive CDKI`s (cyclin dependent kinase inhibitors), in particular p21, as well as in augmented expression of protooncogenes, in particular c-myc. Both mechanisms depend on each other, are procancerogenic and induce enhanced and unarrested proliferation of epithelial cells.

In the present project it was analyzed using an *in vitro* model with the epithelial cell line HEK293T, wether p21 and c-myc in cells with altered LTBP-4-expression undergo a differential expression pattern themselves.

The aim of this project was to get a closer insight into the correlation of the TGF-β binding protein LTBP-4 with the CDKI p21 and the protooncogene c-myc during the carcinogenic process of epithelial cells. Gene silencing as well as transient overexpression of the sLTBP-4-gene in HEK293T cells was established under *in vitro* conditions. The quantitative molecular biological proof of sLTBP-4 overexpression in HEK293T cells through qPCR inquisition was supported by western blot analysis. These proteinbiochemical investigations completed the qPCR survey on a qualitative basis. The sL4-V5 fusion protein was regularly detected, which documents a successful translation.

The following qPCR analysis showed contrary to the expectations that there was no regulation of expression of the genes TGF-β1, p21, and c-myc in the model of sLTBP-4-knock-down. Further on it was expected to detect an enhanced gene expression of TGF-β1 and p21, as well as a reduced expression of c-myc in sLTBP-4-overexpressed cells. In this case the analysis showed an upregulation of TGF-β1- as well as p21-expression and a slightly reduced expression of the c-myc-gene.

The results of this study indicate a correlation of the tested target genes in the model of sLTBP-4-overexpression and clarify their interdependency in the process of carcinogenesis in epithelial neoplasia.