7. Summary

600 candidate genes which are possibly involved in the development of gynecological tumors were identified using an *in silico* approach (eNorthern). Fourty of these candidate genes were selected for further validation within a German research consortium. This dissertation project deals with the validation of two candidate genes, *Tensin* and *SFRP1*. The *in silico* data for *Tensin* and *SFRP1* were confirmed on the RNA level by three independent methods (Northern Blot, quantitative PCR and RNA *in situ* hybridization). Both genes show strongly reduced expression in gynecological tumors compared to normal tissue. Since no aberrations were detected in the coding sequence of both genes, I postulate that the two candidate genes belong to the group of class II tumor suppressor genes.

Tensin is part of focal adhesion complexes, thus playing a role in cell-matrix adhesion as well as in signal transduction processes. The analysis of the *Tensin* expression pattern in mammary gland tissue using RNA *in situ* hybridization revealed an abundant expression in normal breast epithelial cells. Breast tumor cells exhibited a reduced expression or complete loss of *Tensin* in ~50% of all cases investigated in this study. The RNA expression data support the possible involvement of *Tensin* as a tumor suppressor gene in breast cancer development. No mutations were identified in the coding sequence of *Tensin* in genomic DNA isolated from breast tumor tissues. It is conceivable that the *Tensin* inactivation during tumor development is due to epigenetic mechanisms, i.e. promoter hypermethylation. If the loss of Tensin expression is relevant for tumorigenesis or if Tensin can be used as a novel marker in cancer diagnostics, has to be shown by further studies on protein level as well as in cell culture experiments.

The second gene investigated in this thesis, *secreted Frizzled-related protein 1* (*SFRP1*), is a negative regulator of the Wnt pathway. An SFRP1 specific antibody was generated and characterized to analyze SFRP1 expression on protein level and to investigate the association of SFRP1 expression with clinicopathological parameters and patient survival. The analysis of >2000 invasive breast tumors and 56 carcinoma *in situ* revealed similar frequencies of SFRP1 loss in these tumors (46% and 43% respectively). Therefore, I propose that loss of SFRP1 expression is an early event in breast tumorigenesis. SFRP1 expression was inversely correlated with tumor stage ($p<0.001$) but not with other prognostic parameters like tumor grade or lymph node status. Performing a multivariate analysis we could confirm the association between tumor stage and SFRP1 expression ($p=0.029$). In particular, loss of SFRP1 expression in early stage breast tumors (pT1) was associated with poor prognosis

(p=0.04). Functional analyses revealed a possible influence of SFRP1 on the regulation of cell adhesion to the ECM whereas no effect on invasiveness of tumor cell lines was observed in the cell culture model. A possible involvement in proliferation of tumor cell was detectable at certain time points (24h and 48h). In conclusion, loss of SFRP1 is most likely not the initial event directly leading to breast tumorigenesis but facilitating breast cancer development if other genetic changes occur in the cell. Still, SFRP1 expression might be useful as a novel prognostic marker in early stage breast cancer.