7 SUMMARY

Analysis of differential gene expression of the tumour suppressor genes TGF-beta1-3 and its binding proteins LTBP-1, -3 and -4 in canine mammary gland tumours

52 % of the neoplastic diseases occurring by older dogs are tumours of the mammary gland. Various etiological and pathogenic factors are being discussed as a cause of the development of canine mammary gland tumours. Exogenous and endogenous factors are involved. A range of exogenous factors are for example chemical, physical, infectious and viral pathogens. Endogenous factors include especially age, gender, genetic and individual predispositions.

Regarding the carcinogenetic mechanism on the molecular level it is considered that the pathogenesis involves cellular »nonlethal genetic damage« (mutation) of a cell which leads to neoplastic changes through clonal expansion of transformed cells.

Concerning the genetic level three different classes of normal regulatory genes are principle targets of genetic damage. These include »growth promoting protooncogenes«, genes which lead to apoptosis, and the »growth inhibiting tumour suppressor genes«.

The major growth inhibiting genes that are involved in the pathogenesis of canine mammary gland tumours are tumour suppressor genes such as TGF-ß-isoforms (Transforming growth factor-ß1-3) and their binding proteins the LTBP´s (Latent TGF-ß binding proteins 1, 3 and 4).

The aim of this dissertation included an evaluation of clinical data such as gender, age, breeding and neuter status of female dogs suffering from canine mammary gland tumours as well as a pathohistological classification of the excised tumours. Furthermore a quantitative genetic expression analysis of the gene expression profile of the tumour suppressor genes TGF-ß1, 2 and 3 as well as LTBP-1, -3 and -4 was done to evaluate the gene interdependencies in different classified canine mammary gland tumours.

The accomplished data was derived from 45 canine mammary gland tumours of 31 female dogs. Approximately 44 % of the investigated bitches were half-breeds. A general statement concerning the occurrence of canine mammary gland tumours in relation to specific breeds can not be made due to lack of census data. The examined animals had an average age of 9, 5 years and 90, 3 % of them were not neutered. The frequency of the occurring tumours increased from cranial to the caudal mammary gland complexes.

The results of this study show the differential gene expression of the TGF-beta-isoforms 1–3 and the LTBP-isoforms 1, 3 and 4 considering the various stages of tumour progression as well as the different pathohistological classification of canine mammary gland tissue.

A definite tendency in the expression profile of the target genes in adenomas could not be determined regarding correlation and compensation mechanisms of all investigated genes.
In simple carcinomas and complex carcinomas a similar expression pattern of the target genes (TGF-beta1, 2, 3 and LTBP-1, -3 and -4) could be statistically proved. The descriptive analysis showed a downregulation of TGF-beta1, whereas TGF-beta 2 and 3 were apparently not affected. LTBP-1 and LTPB-4 were downregulated and LTBP-3 showed an increased expression in these tumours.
Finally the descriptive and analytical statistics showed various dependencies (correlation and compensation of expression) between following genes regarding simple carcinomas and complex carcinomas:
»TGF-ß1 and TGF-ß2«, »TGF-ß1 and LTBP-1«, »TGF-ß1 and LTBP-3«, »TGF-ß1 and LTBP-4«, »TGF-ß2 and LTBP-3«,»TGF-ß2 and LTBP-4«, »TGF-ß3 and LTBP-4«, »LTBP-1 and LTBP-4« as well as »LTBP-3 and LTBP-4«.
In highly malignant simple carcinomas an increased expression of TGF-beta1, LTBP-1 and LTBP-3 was detected in the expression pattern of the target genes. The TGF-beta-isoforms 2 and 3 stayed unregulated and in addition LTBP-4 was downregulated in highly malignant simple carcinomas.
The determined proportional expression of TGF-beta1 and LTBP-1 and 3 in different classified tumours indicates a correlation of expression of these target genes. This emphasizes the significance of latent TGF-beta binding proteins (LTBP-1 and 3) as modulators of the bioavailability of TGF-beta1.
The discrepancy of the expression of TGF-beta1 in simple and complex carcinomas and highly malignant simple carcinomas confirms the dual role as well as the two-phasical behaviour of the cytokine in the genesis of epithelial neoplasia.
In the early stage of tumour progression the cytokine TGF-beta1 performs as a tumoursuppressor. TGF-beta1 follows the signal transduction pathway and executes its function as a tumoursuppressor indirectly through inhibition of the progression of the cell cycle. In the late stage or in tumours of high malignancy TGF-beta1 promotes as a protooncogene in terms of local invasion and metastasis of tumours.
Epithelial tumours may develop a resistance against the TGF-beta inducted growth inhibition which therefore documents an advanced progression in terms of malignant transformation. Furthermore TGF-beta1 induces an epithelial-to-mesenchymal transition (EMT). After the epithelial tumour converts to a mesenchymal phenotype TGF-beta1 promotes tumourprogression and –metastasis.