Chapter 8

Summary

Although salivary gland tumors represent only a small portion of all human cancers, they are a major challenge to pathologists and clinicians. Their morphologic diversity, with over 40 different histologic subtypes listed, can frequently impose diagnostic problems. Even in the case of a fairly straightforward diagnosis, prediction of their biologic behavior is difficult.

Since current diagnostic and prognostic parameters, consisting mainly of clinicopathological variables, are not always as reliable as desired, many studies attempted to search for markers of disease on a protein level. Focus of these immunohistochemical investigations comprised mostly of known oncogenes or tumor suppressor genes (such as members of essential cell cycle pathways and cell adhesion factors). However, since salivary gland tumors are relatively rare, samples sizes in most of these studies remained small and as a consequence, results were sometimes difficult to interpret. Another limitation of these tumors is the necessity for an extensive follow-up time; recurrences and metastases can develop even after 20 years of diagnosis. Because of the lack of such long time follow-up data, many studies do not possess either reliable or even any prognostic significance.

One cellular component of salivary gland tissue, the myoepithelial cell, has received increasing attention in recent years because of its natural tumor suppressive phenotype. Albeit myoepithelial cells tend to resist transformation, neoplastic myoepithelial cells are a main component of various salivary gland tumors. The complex histologic and biologic characteristics of these myoepithelial cells are also mirrored in the broad morphology and clinical behavior of the tumors they produce. Unfortunately, while it is believed that myoepithelial salivary gland carcinomas, like other neoplastic lesions, develop and progress through an accumulation of alterations in proto-oncogenes and tumor-suppressor genes, genetic events leading to the initiation and progression of these cancers are poorly characterized. Immunoprofiling and genomic analysis of a relatively large group of myoepithelial neoplasms could significantly improve their classification and prognostication.

To this end, the main goal of the research described in this thesis was to evaluate disturbances on a protein and chromosomal level in
salivary gland tumors with myoepithelial differentiation. We aimed to extend our knowledge on (1) the expression of important proteins involved in cell cycle regulation and maintenance of cell identity and (2) the chromosomal aberrations that underlie tumor development.

In chapter 2, it is shown in a relatively large group of adenoid cystic carcinomas (ACCs; \( n=18 \)) that this malignancy harbors a large amount of genomic aberrations, in which DNA copy number increases predominate. The most frequent gains include numerous loci of growth factors and their receptors, especially fibroblastic growth factors (FGFs). Notably, gains at loci of growth factors occur more frequently in aggressive ACCs compared to indolent tumors. Survival correlates with the number of genomic aberrations in this neoplasm.

In chapter 3, the expression of the proteins p16\(^{\text{INK4a}}\), E2F1, Cyclin D1, p53 and Ki-67 is determined in 21 paraffin-embedded ACCs by immunohistochemistry. Compared to normal salivary gland tissue, expression levels of all proteins examined are significantly elevated in ACC. In this group of tumors, low Cyclin D1 staining correlates with the occurrence of metastasis during the time of follow-up. Normal salivary gland tissue exhibits a mutually exclusive expression pattern of the two Polycomb group (PcG) complexes. In ACC, this pattern is greatly disturbed, with EZH2 and EED being highly expressed in the presence of BMI-1 and MEL-18. High EZH2 expression is predictive of unfavorable outcome in univariate analysis. Multivariate analysis reveals the presence of recurrence as the most important prognostic factor.

In chapter 4, a limited amount of genomic aberrations is described in both benign (\( n=15 \)) and malignant (\( n=12 \)) myoepitheliomas. Frequent DNA copy number changes are detected at loci of growth factors and growth factor receptors (PDGF, EGFR and several FGFs), and common losses are observed at loci of proto-cadherins. Unsupervised clustering reveals separate clusters with a clear correlation to malignancy.

In chapter 5, protein expression levels of the cell cycle regulators p16\(^{\text{INK4a}}\), p53, Ki-67, E2F1, and Cyclin D1 is investigated in a large group of benign (\( n=49 \)) and malignant (\( n=30 \)) paraffin-embedded
myoepitheliomas by immunohistochemistry. In benign tumors, overexpression of Rb-p16 pathway members is present. In addition to this deregulation, malignant myoepitheliomas show overexpression of p53. The five recurrences of histologically benign tumors in this group show similar immunoprofiles as the carcinomas by demonstrating deregulation in both pathways. As in normal salivary gland tissue, a mutually exclusive expression pattern of the two PcG group complexes is observed in the benign tumors while malignant myoepitheliomas overexpress EZH2.

Finally in chapter 6, the genomic profile of a third type salivary gland tumor with myoepithelial differentiation is investigated, namely a carcinosarcoma. In this study, a high-resolution oligonucleotide array is used to examine the genomic profiles of the two distinct components in the tumor separately. Both elements show a high number of alterations, which are shared for 75%. The extensive overlap between the two profiles indicates a monoclonal origin for the two components.