4. Fonseca I, Soares J. *Epithelial-myoepithelial carcinoma of the salivary glands. A study of 22 cases.* Virchows Archiv A Pathol Anat 1993; 422: 389-396.
5. Rosa JO, Fonsecs I, Fblix A at al. *p53 immunoexpression in carcinomas arising in pleomorphic adenomas.* mt J Surg Pathol 1996; 3: 257-262.

Molecular biology and immunohistochemistry

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Several salivary tumors show microscopically recognizable subtypes, which may be related to the grade of malignancy. It is tempting to believe that the relatively poor prognosis higher grade subtype arises from one or more of the lower grade subtypes via a series of consecutive, partly independent, molecular progression events in certain genes. The most commonly involved gene families in epithelial malignancies are *p53, myc, ras,* and *neu,* whilst *raf, myb, fur and lun,* and genes that code for epidermal growth factor-R (EGF-R) and platelet-derived growth factor (POGE) are less common. Pleomorphic adenoma (PA) is characterized by recurrent chromosome rearrangements, reciprocal translocations in particular, with breakpoints at 8q12, 3p2I, and 12q13-15. The most common abnormality in PA is a reciprocal t(3;8)(p21 ;q21); this results in promoter swapping between the gene for Il-catenin and PLAGi, a novel zinc finger gene at 8q12 (1). This finding indicates that PLAGi activation due to promoter swapping is very likely to be a crucial event in salivary gland tumourigenesis.

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The presence of p53 gene abnormalities has been analyzed [bothby polymerase chain reaction (PCR)-based assays and immunohistochemistry (IHO)] in *e.g.,* PA and carcinoma in pleomorphic adenoma (CPA). Loss ot heterozygosity (LOH) of the p53 gene occurs in approximately 50% of PAs and in more than 85% of CPAs. The p53 oncoprotein is overexpressed in some 10% of adenomas and 75% of carcinomas, and tumors with overexpression of p53 protein demonstrate LOH. Investigation (fluorescence *in situ* hybridization) of numeric aberrations of chromosome 17 in PA and CPA revealed that loss of chromosome 17 may occur in PA betore its transformation to CPA. p53 expression is frequently associated with deletion of the p53 gene, and polysomy 17 is more frequent in CPA than PA, and associated with mutation of p53. In agreement, other reports suggest that alterations on the short arm of chromosome 17 may represent an event related to salivary gland tumor progression.

Adenoid cystic carcinomas (AdCC) also show a relatively high incidence of LOH at the p53 gene, and also at the Rb gene. The number of mutations (both LOH and microsatellite alterations) at either gene is greater in histologically high-grade foci than low. AdCCs show no K-ras mutations at the sites suspected of harboring recessive oncoganes. In contrast, a single type of p53 point mutation has been demonstrated in recurrent AdCCs, but not in the primaries (tissue PCR amplified for p53 exons S to 8, and direct sequencing). The presence of p53 oncoprotein positive tumor cells and the point mutations in recurrent AdCC suggest that p53 alterations are involved in late rather than early stages of tumor progression. In dedifferentiated acinic cell carcinoma, both low- and high-grade components were negative for p53 oncoprotein expression, and POR and single-strand conformation polymorphism analyses were consistent with a germ line configuration of the p53 gene in both elements of this tumor. In agreement, p53 protein accumulation seems to be unrelated to tumor recurrence (2). These findings suggest that p53 is far from always an early event in the malignant transformation and progression of salivary gland tumors.

myc. ras. Rb. c-erbB and others

The synergistic interaction of the amplified *c-myc* oncogene with transformation growth factor-ct (TGF-ct) *in vitro* to promote phenotypic transformation of mammary epithelial cells, apparently also takes place in mouse salivary gland tumorigenesis. Investigation of salivary adenocarcinomas for mutational activation of H-ras, K-ras and N-ras has shown an unusual ras mutation in 3 of 13 cases (A:T to G:C transition at the second position of codon 51 of the *H-ras* gene). C-erbB-2 oncoprotein is rarely expressed in salivary tumors. Bcl-2 is present in a wide variety of non-Hodgkin's lymphomas and the oncoprotein is also expressed in several salivary carcinomas, and reactive conditions of salivary gland mucosa-associated lymphatic tissue. It was recently shown that PAs have few areas of allelic loss, and that many salivary carcinomas displayed allelic loss patterns different from many other human tumor types (3). This latter finding and the diversity in p53 mutations (see above), suggest distinct genetic pathways in the progression of salivary gland tumors.

Immunohistochemistry

Single and coexpression of many gene products have been studied in relation to histological type, histogenesis, tumor stage, cell proliferation, etc.. Reduced expression of E-cadherin in AdCC correlates with unfavorable prognosis (4), while *e.g.,* expression of oncoprotein p53 in carcinomas appears to be unrelated to prognosis. The few typical' immunoprofiles of some salivary gland tumors are present usually only when the diagnosis already can be made on ordinary hematoxylin and eosin stained sections. Certain tumors have cells that are positive for prostate-specific antigen, which can cause erroneous diagnosis of metastatic disease. Salivary duct carcinoma often expresses c-erbB-2 protein, is positive for androgen receptor and gross cystic disease fluid protein-is, and is usually estrogen and progesterone receptor negative, but this immunophenotype does not totally exclude matastatic breast cancer. Assessment of proliferation by Ki-67 can be of value, *e.g.,* in differentiating difficult cases of AdCC from polymorphous low-grade adenocarcinoma. IHC has limited use in diagnostic salivary pathology and is best appreciated in relation to differentiating primary *vs.* metastatic disease, and in certain rare tumors such as small cell neuroendocrine carcinoma, primitive neuroectodermal tumors, sarcomas, etc.

References

1. Kas K, voz ML, Roiier Eat al. *Promoterswapping between the genes bra novel zinc finger protein and beta-catenin in pleomorphic adenomas with t(3;8)(p21;q12) translocations.* Nat Genet 1997; 15: 170-174.
2. Rosa JO, Fblix A, Fonsaca I at al. *Immunoexpression of c-erba-2 and p53 in benign and malignant salivary neoplasms with myoepithelial differentiation.* J Olin Pathol 1997; 50: 661 -663.
3. Johns MM 3rd, Wastra wH, calitano JA at al. *Allelotype of salivary gland tumors.* Cancer Ras 1996; 56: 1151-1154.
4. Franchi A, Gallo O, Bocciolini C at al. *Reduced E-cadherin expression correlates with unfavorable prognosis in adenoid cystic carcinoma of salivary glands of the oralcavity* Am J Olin Pathol 1999; 111: 43-50.