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Allelotype of Salivary Gland Tumors[1]

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ABSTRACT

To elucidate the genetic alterations that occur in salivary gland tumors, we screened every autosomal arm (and the X-chromosome) of 29 primary human salivary gland neoplasms (11 pleomorphic adenomas, 10 adenoid cystic carcinomas, 5 mucoepidermoid carcinomas, and 3 carcinoma ex-mixed tumors) for allelic loss using 86 microsatellite markers. A minimum of two microsatellite markers were used per chromosomal arm to achieve informativity of at least 60% (excluding X). The pleomorphic adenomas demonstrated few areas of allelic loss; the most prominent chromosomal arm involved was 12q, lost in more than 35% of informative cases. The most significant allelic losses in adenoid cystic carcinoma were 1p, 2p, 6q, 17p, and 20p (> 20% of informative cases) and 19q (40% of informative cases). Mucoepidermoid carcinoma showed 50% or greater loss at 2q, 5p, 12p, and 16q. Although losses at 9p, 3p, and 17p are common in squamous cell carcinoma of the head and neck, only the carcinoma ex-mixed tumors demonstrated loss at these loci, consistent with progression to a more aggressive phenotype. Salivary gland tumors display allelic loss patterns different from many other tumor types, suggesting distinct genetic pathways in the progression of these tumors.

INTRODUCTION

Salivary gland tumors are estimated to occur with an incidence of 1 to 2 per 100,000 Americans each year, representing 1% of head and neck neoplasms (1–3). Approximately 80% of all salivary neoplasms originate in the parotid gland, 10 to 15% arise from the submandibular glands, with the remaining stemming from the sublingual and minor salivary glands (1–3). Malignant tumors represent about 10 to 20% of total parotid gland neoplasms, 40 to 60% of total submandibular neoplasms, and up to 80% of those arising in the sublingual and minor salivary glands (1–4). The pleomorphic adenoma is the most commonly occurring benign tumor of the salivary glands (1–3). Of the malignant tumors, mucoepidermoid carcinoma and adenoid cystic carcinoma occur most commonly (1–3). Pleomorphic adenomas, mucoepidermoid carcinomas, and adenoid cystic carcinomas were selected for this study. Three carcinoma ex-mixed tumors were included to evaluate progression from the benign to malignant phenotype. Carcinoma ex-mixed tumors are thought to represent the malignant evolution of pleomorphic adenomas (4).

Little is known about the genetic alterations that are involved in the development of these tumors. Cytogenetic data implicate chromosome 8q12 rearrangements and chromosome 12q13–15 rearrangements in about 50 and 20% of pleomorphic adenomas, respectively (5–7). Recent studies highlight chromosome 11q14–22 rearrangements in mucoepidermoid carcinoma and 6q21–24, 9p13–23, and 17p12–13 rearrangements in adenoid cystic carcinoma (8, 9). The c-*int* protooncogene (chromosome 12q) was implicated in salivary gland tumors when transgenic mice expressing the *int-1* gene were found to form salivary and mammary gland neoplasms (10). Despite these initial clues, little is known about the biological behavior of these tumors, and analysis by light microscopy is often inadequate in predicting clinical behavior.

To investigate further the genetic aberrations in salivary gland tumors and uncover areas of chromosomal loss that may contain tumor suppressor genes, we performed a comprehensive allelotype of 11 pleomorphic adenomas, 10 adenoid cystic carcinomas, and 5 mucoepidermoid carcinomas. Furthermore, we evaluated three carcinoma ex-mixed tumors at selected loci to look for progression from their benign counterpart. Pleomorphic adenomas showed the highest loss at chromosome 12p (35% of informative cases), adenoid cystic carcinoma at 19q (40% of informative cases), and mucoepidermoid carcinoma showed 50% or greater loss at 2q, 5p, 12p, and 16q. The mixed malignant tumors analyzed demonstrated allelic loss at chromosome 19q in all three cases, suggesting involvement of this arm in the progression to malignancy from their benign pleomorphic adenoma counterpart.

MATERIALS AND METHODS

Tissue and DNA Extraction. Twenty-nine primary salivary tumors were accumulated either from fresh tissue or from banked, paraffin-embedded tissue following surgical resection at Johns Hopkins Hospital. Consent was obtained preoperatively from all patients. Fresh tissue was frozen immediately postoperatively and was meticulously microdissected on a cryostat in the presence of an experienced pathologist (W. W.) for neoplastic cells. Tumors with less than 60% neoplastic cells were excluded from the study. Paraffin-embedded tissue was similarly microdissected but were placed in xylene for 24 h at 48°C to remove paraffin. For each tumor, more than 50 12-μm sections cut were obtained and placed in SDS/proteinase K for 24 h at 48°C. Samples were subjected to phenol-chloroform extraction and ethanol precipitation as described previously (11). Matched normal DNA was obtained from isolation of lymphocyte DNA or microdissection of normal nonneoplastic tissue obtained at surgery. Extraction of DNA was performed as described above. Clinical and histopathological classifications were based on American Joint Committee on Cancer staging (12).

Allelotyping. Microsatellite markers designed for PCR were obtained from Research Genetics (Huntsville, AL). Markers used were as follows: chromosome 1p, *D1S162* and *D1S219;* 1q; *D1S158* and *D1S117;* 2p, *D2S162* and *D2S244;* 2q, *D2S126* and *D2S125;* 3p, D3S1067, D3S1284, and D3S1038; 3q, *D3S1238* and *D3S1292;* 4p, *D4S404* and *D4S1546;* 4q, *D4S1613* and *D4S171;* 5p, *D5S392* and *D5S417;* 5p, *D5S421* and *IL-9;* 6p, *D6S273* and *D6S287;* 6q, *D6S268* and *D6S287;* 7p, *D7S481* and *D7S507;* 7q, *D7s522, D7S677, D7S479,* and *D7S486;* 8p, *D8S261* and *D8S262;* 8q, *D8S273* and *D8S167;* 9p, *IFN-α, D9S736, D9S171,* and *D9S2230;* 9q, *D9S12* and *D9S109;* 10p, *D10S226* and *D10S249;* 10q, *D10S221* and *D10S185;* 11p, *D11S899* and *D11S907;* 11q; *INT-2, D11S873,* and *PGYM;* 12p, *D12S62* and *D12S77;* 12q, *D12S95* and *D12S60;* 13q, *D13S170* and *D13S133,* 14q, *D14S256* and *D14S51;* 15q, *D15S117* and *D15S87;* 16p, *D16S404* and *D16S418;* 16q, *D16S402* and *SPN;* 17p, *TP53* and *CHRNB-1;* 17q, *D17S579* and *D17S250;* 18p, *D18S40* and *D18S59;* 18q, *D18S34* and *DCC;* 19p, *D19S177;* 19q, *D19S210* and *D19S246;* 20p, *D20S95* and *D20S116;* 20q, *D20S101* and *D20S119;* 21q, *D21S223* and *d21S417;* 22q; *D22S2282* and *IL2RB* and X, *DXS451, DXS1068,* and *DXS1108.* Markers were selected to ensure at least 60% informativity for all cases on every chromosomal arm. Fifty ng of one primer from each pair was end-labeled with [^{32}P]ATP (20 mCi; Amersham) and T4 kinase (GIBCO-BRL) in a total volume of 50 μl. Ten μl PCR reactions were performed using 25 ng of genomic DNA, 0.5 ng of end-labeled primer, and 75 ng of each unlabeled primer. PCR conditions were as follows: 30–35 cycles of 95°C for 30 s

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(denaturation), 50–60°C for 1 min (annealing), then 70°C for 1 min (extension) as described previously (13). Three μl of each PCR product were separated on an 8% urea-formamide-polyacrylamide gel and exposed to film for 12 to 24 h as described. (14) Allelic losses were scored for informative cases if one allele in tumor DNA was visually more than 50% reduced in intensity when compared to the same allele in the normal DNA.

RESULTS

Twenty-six primary human salivary gland tumors (11 pleomorphic adenomas, 10 adenoid cystic carcinomas, and 5 mucoepidermoid carcinomas) were screened for LOH[3] at every autosomal arm with 86 informative microsatellite markers. (Additionally, three carcinoma ex-mixed tumors were studied with a subset of the markers used to screen the benign pleomorphic adenomas to evaluate genetic progression.) Representative results are displayed in Fig. 1 where tumor S5 has lost the upper allele at markers *D6S287* and *D6S268*, tumor S12 has lost the lower allele at marker *D6S268*, and tumor S7 has lost the upper allele at marker *D6S262*.

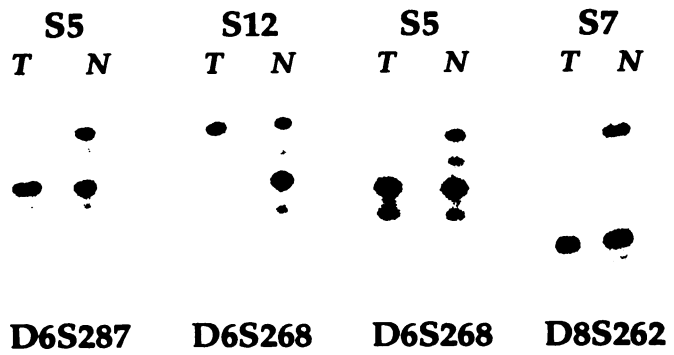

Fig. 1. Autoradiographs depicting LOH analysis with microsatellite markers. Representative salivary gland tumors (*T*) and corresponding normal tissue (*N*) are shown with microsatellite markers indicated on the *bottom*. From *left* to *right:* tumor S5 exhibits loss of upper allele with marker *D6S287*. Tumor S12 exhibits loss of lower allele with marker *D6S268*. Tumor S5 exhibits loss of upper allele with marker *D6S268*. Tumor S7 exhibits loss of upper allele with marker *D8S262*.

Results for these three subtypes of salivary gland tumors are shown in Fig. 2. Adenoid cystic carcinoma showed allelic loss in 40% of informative cases on chromosome 19q. Moreover, chromosomes 1p, 2p, 6q, 17q, 19p, and 20q demonstrated loss in 20 to 30% of informative cases. LOH on other arms was less frequent. Fig. 2 also depicts the results for mucoepidermoid carcinoma. These tumors displayed loss in 50 to 60% of informative cases at chromosome 2q, 5p, 12p, and 16q. Chromosomes 5q and 11q were lost in 40% of informative cases. Pleomorphic adenomas (Fig. 2) revealed LOH in more than 35% of informative cases at chromosome 12q. Chromosome 5p, 11q, and 19q had greater than 20% loss, while other arms were involved less frequently. MAL was calculated as the mean number of chromosomal arms lost per tumor for each histopathological type. Mucoepidermoid carcinoma had a MAL of 6.03 (+/− SD 3.57). Adenoid cystic carcinoma and pleomorphic adenoma were found to have MALs of 2.40 (+/− SD 1.49) and 2.26 (+/− SD 1.42), respectively.

To evaluate progression from benign to malignant phenotype, we screened three carcinoma ex-mixed tumors with a subset of microsatellite markers. The initial benign adenomas from these patients were not available for analysis. The markers selected covered chromosome 9p, 3p, and 17p (frequently lost in other head and neck tumors) and the chromosomal arms that displayed any loss in the benign pleomorphic adenomas (5p, 7q, 10p, 11q, 12q, 19q, and 22q). Chromosome 9p was lost in 3 of 3 cases, 3p showed LOH in 2 of 3 cases, and 17p had allelic loss in 1 of 3 cases. The tumors were tested with other markers and demonstrated loss of chromosome 11q and 19q in 2 of 2 cases, 10p in 2 of 3 cases, and 5p and 7q in 1 of 3 cases. No allelic loss was observed on chromosomes 12q and 22q.

DISCUSSION

Neoplasia occurs through the accumulation of multiple genetic alterations (17). Loss of functional tumor suppressor genes is associated with chromosomal deletions and appears critical to the progression of human cancer. Originally a hallmark of colon cancer progression (16, 17), these deletions have been found in many other tumor types, including squamous cell carcinoma of the head and neck, lung, breast, bladder, prostate, and brain tumors (18–23). We have completed a comprehensive allelotype, screening every autosomal arm and the X-chromosome for LOH, in the major subtypes of human salivary gland tumors. Regions of chromosomal loss found in these tumors can help identify potential sites for tumor suppressor genes. Although some regions of loss are shared with other tumor types, many of these regions appear more unique to salivary gland tumors, suggesting distinct genetic pathways in the genesis of these neoplasms.

Adenoid cystic carcinoma is characterized by loss of chromosome 19q in 40% of informative cases. This locus may be the site of an important tumor suppressor gene since chromosome 19q loss is also a significant feature of human brain tumors (24). Cytogenetic studies of adenoid cystic carcinoma have pointed to chromosomal translocations and deletions at 6q21–24 and translocations of 9p and 17p (9). Although we did not observe loss at 9p, LOH at 6q and 17p is consistent with these observations.

Mucoepidermoid carcinoma, in general, demonstrates a higher rate of allelic loss. Chromosomes 5p and 16q demonstrate allelic loss in 60% of informative cases, and these loci have been implicated in prostate cancer (16p), cervical cancer (5p), and ovarian cancer (16p) (25–27). Chromosome 12p loss (50% of informative cases) has been observed in germ cell testicular cancer (28).

Pleomorphic adenomas, like adenoid cystic carcinomas, demonstrate relatively infrequent LOH. The chromosomal arm showing the most frequent allelic imbalance is 12q (more than 35% of informative cases). Given the observation that INT-1 transgenic mice develop salivary gland neoplasms (10), this allelic imbalance may actually represent an amplification and may harbor a positive regulator of tumorigenesis. Chromosome 12q is also lost in 33% of human ovarian tumors (27), and translocations involving chromosome 12q have been observed in cytogenetic studies in human pleomorphic adenomas (5–7). Recurrent rearrangements in the high mobility group protein gene (*HMGI-C* on chromosome 12q) have been noted in benign mesenchymal tumors as well as pleomorphic adenomas (29). We observed LOH in 18% of informative cases on chromosome 7q. Evidence for a broad range tumor suppressor gene located at 7q31.1 is strongly suggested in studies of prostate cancer, breast cancer, head and neck squamous cell carcinoma, and colon cancer and may be important in pleomorphic adenomas (30–32).

We elected to analyze three carcinoma ex-mixed tumors at selected loci to evaluate progression from their benign counterparts. The most striking difference is loss of 3p, 9p, and 17p in these mixed malignant tumors in contrast to the absence of loss at these loci in the pleomorphic adenomas. Although the sample size is small, this observation suggests the importance of 3p, 9p, and 17p involvement in progression to a more malignant phenotype. Loss at 3p, 9p, and 17p are very

[3] The abbreviations used are: LOH, loss of heterozygosity; MAL, mean allelic loss.

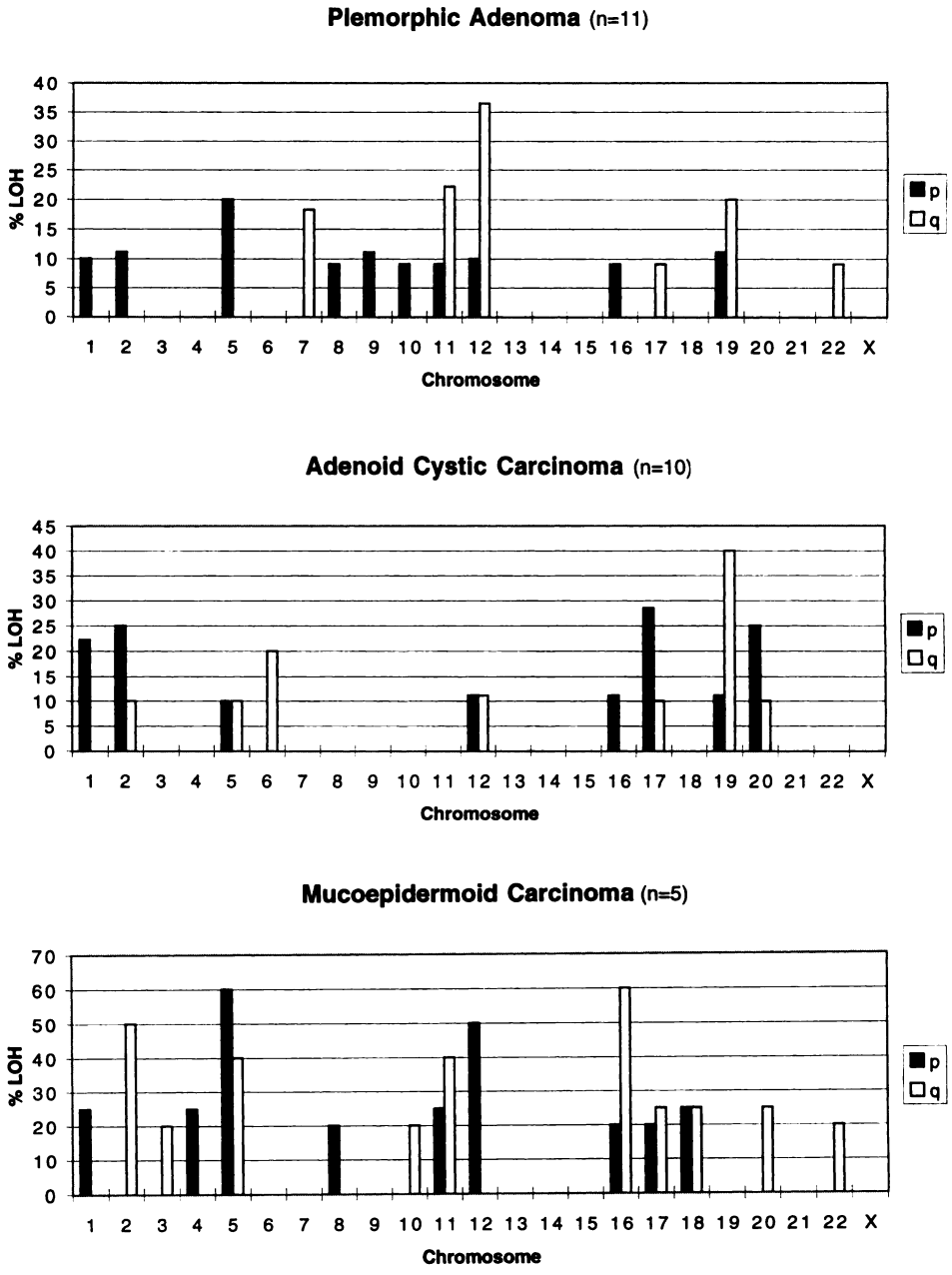

Fig. 2. Frequency of allelic loss for chromosomes in pleomorphic adenoma, adenoid cystic carcinoma, and mucoepidermoid carcinoma. Markers used are listed in "Materials and Methods." The three carcinoma ex-mixed tumors are not shown.

common alterations seen in many clinically aggressive epithelial tumors and may be markers for more malignant progression in these tumors. The carcinoma ex-mixed tumors showed LOH on several chromosomal arms (5p, 7q, 10p, 11q, and 19q) also lost in benign pleomorphic adenomas, suggesting similar background genetic alterations. Although inconsistencies in LOH on chromosome 12q and 22q (loss in 0 of 3 carcinoma ex-mixed tumors) are most likely due to the small sample size of the carcinoma ex-mixed tumors, we cannot rule out the possibility that these tumors might arise from different cells of origin.

We have completed a comprehensive allelotype of human salivary gland tumors. Our data demonstrate allelic loss at sites consistently seen in other tumor types but also implicate new loci that may harbor suppressor loci more uniquely involved in the tumorigenesis of salivary gland neoplasms. Moreover, we have demonstrated different patterns of deletion between various salivary gland tumor types. This observation seems to give molecular support to the previously described histopathological differences that suggest a different origin and development for each of these tumor types. More studies must be performed on these neoplasms to further understand the critical steps involved in tumor development. This molecular information may then be used to develop novel diagnostic and therapeutic approaches for affected patients.

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