Combined Strategy of Conventional Cytogenetics, Fluorescent In Situ Hybridization and Chromosome Morphometry for Analysis of Parotid Gland Tumor*

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ABSTRACT

The limiting factors in conventional cytogenetic analysis of cell culture, especially of solid tumors, include insufficient metaphases, overgrowth of abnormal mitotic cells by normal cells, and suboptimal quality of harvesting and banding. Despite the availability of numerous protocols to induce G-banding, as well as Q-, R-, and C-banding, occasions still arise in which the analysis is severely limited by these factors and incomplete conclusions are often drawn as to the precise nature of the chromosomal abnormality, if indeed any can be detected. By adopting a rational approach of (1) close monitoring of cultures and rapid harvesting as soon as it is feasible, and (2) analysis of available metaphases by a combination of the GTG technique, fluorescent in situ hybridization (FISH), and chromosome morphometry using a graphic arts tool, a significant improvement in success rate may be more readily achieved. Here pathological and cytogenetic data are presented of a case of parotid gland carcinoma ex mixed tumor with the karyotype of 46, XX, del(5)(q12), dir ins(8;5)(q12;q12qter), add(12)(p13)/46,XX. This case is utilized to illustrate the importance of application of our combined strategy.

Introduction

Progress in solid tumor cytogenetics has been hampered by failure to establish a culture, a low mitotic index, an
overgrowth of abnormal cells by normal fibroblasts, fuzzy chromosome morphology and suboptimal banding. As with many other tumors, the number of cases of salivary tumors studied cytogenetically is disappointingly small.

The majority of cytogenetic studies in the literature on salivary gland neoplasms have involved the benign mixed tumor. Two groups, those of Mark in Sweden and Bullerdiek in Germany are responsible for most of the recent studies on mixed tumors. Clonal chromosome abnormalities, such as reciprocal translocations or deletions, were found in approximately 50 percent of tumors. Patterns of recurrent, specific, and mutually exclusive structural rearrangements that involve chromosomes 8 and 12 have been identified that may define certain distinct clinicopathologic features of mixed tumors. However, the information currently available is fragmented and incomplete. A need thus exists for additional comprehensive cytogenetic studies of salivary tumors using innovative approaches to determine their pathogenetic significance.

Clinical History

The patient was a 53-year-old female with a 3 × 3 cm painless mass in her left parotid gland, which had been increasing in size over the past few years. There was no cervical adenopathy and the seventh nerve was intact. Her previous medical history was unremarkable. A left superficial parotidectomy was performed. The patient is currently alive and well 13 months after surgery with no evidence of tumor.

Materials and Methods

CONVENTIONAL CYTOGENETICS

Samples of tumors were carefully minced and grown in tissue culture media. Cultured cells were closely monitored. Colcemid (deacetylmethylcolchicine at 10 μg/ml) was added to tissue culture flasks approximately 1.5 hours prior to harvest. Harvest for routine tissue culture was performed analogous to the lymphocyte culture technique of Moorhead et al using 75 mM KCl as the hypotonic agent and three parts of methanol to one part of glacial acetic acid as the fixative. Air-dried slides were prepared after repeated rinsing and centrifugation. GTG-banding (Sumner et al) was induced using trypsin (Seabright). Slides were subsequently stained in 4 percent Giemsa (Gurr's improved R66), and dried.

FLUORESCENT IN SITU HYBRIDIZATION

Fluorescent in situ hybridization (FISH) was performed according to detailed methods described in Mark et al and Mark. For this application, chromosome 8 and chromosome 12 painting probes were procured commercially.

CHROMOSOME MORPHOMETRY

Chromosome morphometry was performed according to Mark et al using Nestler Run-Mate a graphic arts tool with a built-in calculator.

STATISTICAL ANALYSIS

Statistical analysis was performed using StatView II SE & Graphics Statistical Program.
Pathologic Findings

The tumor measured 2.5 cm in diameter, was yellowish in color, rubbery in consistency, and surrounded by normal tissue. Histologically the tumor was an invasive carcinoma ex mixed tumor with a moderately differentiated adenocarcinoma that invaded through the capsule in several areas. Surgical margins were free of tumor.

Cytogenetic Results and Discussion

Chromosomal analysis of 20 metaphases derived from tissue culture of this parotid gland specimen, harvested 17 days after specimen receipt, revealed the modal cell line to be pseudodiploid with the following apparent karyotype: 46, XX, del (5) (q12), dir ins (8;5) (q12; q12qter), add (12) (p13)/46,XX. Only q12 vs q13 one cell in this sample was found to be apparently normal diploid, within the limits of the technology utilized in this study. A representative GTG-banded abnormal cell is shown in figure 1. Although the morphology of the chromosomes based on GTG-banding indicated breakage at the q12 region of chromosome 5 and insertion of chromatin material from q12 to the terminal region of the

![Figure 1](image.png)

**Figure 1.** The GTG-banded karyotype of an abnormal cell from the initial harvest. Longer arrows point to abnormal markers resulting from apparent insertion of chromosome 5 material into chromosome 8. Shorter arrow points to an abnormal chromosome 12 homolog with additional chromatin material on its short arms.
long arms of chromosome 5 into chromosome 8, the possibility of an interstitial deletion extending from 5q12 to a subtelomeric band on 5q cannot be ruled out. In fact, it is highly likely that the derivative chromosome 5 is capped by telomeric sequences (Müller16).

Eight weeks after receipt, another harvest was performed to obtain additional slides for FISH "as the supply of available slides was exhausted". Fluorescent in situ hybridization with a chromosome 8 and a chromosome 12 painting probe (data not shown) revealed all cells to be apparently normal at this passage. Thus, the previously minor normal subpopulation has overgrown the abnormal clone. This phenomenon suggests there is a rather narrow window of opportunity for study, which may pose difficulties in terms of attaining sufficient numbers of mitotically active cells from the neoplastic clone for analysis without significant protocol modifications. The need for rapid harvest, as illustrated here, cannot be overemphasized.

By using a novel morphometric approach, yet another adjunct technique to corroborate results of conventional cytogenetics can be provided. Chromosome morphometry was performed on GTG-banded karyotypes according to guidelines as established in Mark et al.13,14,15 Morphometric studies performed on four complete karyotyped cells yielded relative length values for each chromosome of the genome. It was hypothesized that for chromosomes 5 and 8, if there was indeed a direct insertion of chromosome 5 material into chromosome 8 with no appreciable loss (or gain) of chromatin material, then the sum of the relative lengths of the two reciprocal products should be equal to the sum of the normal chromosome 5 and normal chromosome 8 homologs. The two reciprocal products of the postulated structural rearrangement are der(5), which is the smaller of the two marker chromosomes, and der(8), which is the larger of the two marker chromosomes. A paired 2-tailed t-test, carried out to test the null hypothesis of no difference between the average means of chromosome 5 plus chromosome 8 versus der(5) plus der(8), yielded a p value of .72 (with 3 degrees of freedom) which is not significant. This result is consistent with the null hypothesis and our postulated scheme for the chromosomal structural rearrangement.

In contrast, when relative length (RL) values of the apparently normal chromosome 12 and the chromosome 12 with apparent additional material in the short arms are compared, a p value of 0.05 (with 3 degrees of freedom) was obtained. This finding supports the alternative hypothesis of an abnormal chromosome 12 with additional material on the p13 region, as postulated. It is of interest to note that excess copy number of chromosome 12p13 material has been reported in other tumors17 although the exact significance of the amplification of these 12 p sequences in tumor evolution and progression is not known.

As previously mentioned, progress in solid tumor cytogenetics has been hampered by culture failure, low mitotic index, overgrowth of abnormal cells by normal fibroblasts, fuzzy chromosome morphology and suboptimal banding. With the advent of fluorescent in situ hybridization, the nature of otherwise unidentifiable marker chromosomes can be more readily delineated and interphase as well as metaphase cells are amenable to analysis. The rate of success in tumor cytogenetics can be significantly increased by adopting a rational, combined approach of the following: (1) speed processing to decrease loss of viable cells, (2) close monitoring of cultures, (3) rapid harvest to prevent selective in vitro elimination of the abnormal clone, (4) analysis using FISH together with
GTG-banding and CBG staining, and (5) corroboration of conventional and molecular cytogenetic data with chromosome morphometry, which in our case was achieved conveniently and inexpensively by adopting a novel device.

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