Pergamon

Oral Oncol, Eur J Cancer, Vol. 32B, No. 2, pp. 128–132, 1996

Printed in Great Britain
0964-1955/96 $15.00 + 0.00

0964-1955(95)00078-X

Malignant Salivary Gland Neoplasms: a Cytogenetic Study of 19 Cases

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A group of 19 malignant salivary gland neoplasms of various histological types (mucoepidermoid carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, epithelial–myoepithelial carcinoma, myoepithelial carcinoma, basal cell adenocarcinoma, carcinoma ex-pleomorphic adenoma, ductal carcinoma, adenocarcinoma not otherwise specified and undifferentiated carcinoma) were cytogenetically investigated. Previous karyotypic information revealed deletion of the long arm of chromosome 6, loss of chromosome Y and the gain of chromosome 8 as the most recurrent deviations found in these neoplasms. Clonal chromosome aberrations were detected in 11 cases of this series. In 7 of them there were only numerical deviations (gain of chromosomes 2, 7, 8, 10 and X and loss of chromosomes 18, 21 and Y) without concomitant structural anomalies. Structural rearrangements such as t(2;7), t(6;16), t(6;9) and t(1;1) translocations were found in two mucoepidermoid carcinomas, one adenoid cystic carcinoma and one ductal carcinoma, respectively.

The wide spectrum of changes found in this group of neoplasms may reflect the diversity in their histogenesis and differentiation phenotypes. Copyright © 1996 Elsevier Science Ltd

Keywords: salivary gland neoplasms, cytogenetics, adenoid cystic carcinoma, mucoepidermoid carcinoma

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INTRODUCTION

Malignant salivary gland tumours are infrequent neoplasms that present various morphological patterns as well as diverse clinical behaviour [1, 2]. Cytogenetic studies on salivary gland tumours are almost restricted to benign tumours, especially pleomorphic adenomas [3–5]. The information regarding the chromosomal patterns of malignant salivary gland neoplasms is rather limited [4–17]. Structural rearrangements involving the long arm of chromosome 6, loss of chromosome Y and gain of chromosome 8 are recurrent clonal deviations that have been demonstrated in these tumours [4–17].

We present herein additional cytogenetic information on the frequency and specificity of chromosomal deviations found in a series of 19 consecutive malignant salivary gland tumour types of diverse histological type.

MATERIALS AND METHODS

Study population

Nineteen malignant salivary gland tumours were successfully processed for cytogenetic study. The patient's age ranged between 12 and 79 years (Table 1). All the tumours were located at the major salivary glands (parotid gland $n=15$; submaxillary gland $n=3$), but case 8 originated in the minor salivary glands of the oral cavity.

Methods

The neoplasms were classified according to the World Health Organization classification [2] into the following categories: mucoepidermoid carcinomas ($n=5$), adenoid cystic carcinomas ($n=3$), carcinomas ex-pleomorphic adenoma ($n=3$), epithelial–myoepithelial carcinomas ($n=2$) (Fig. 1); acinic cell carcinoma ($n=1$), adenocarcinoma NOS (not otherwise specified) ($n=1$), basal cell adenocarcinoma ($n=1$), undifferentiated carcinoma ($n=1$), myoepithelial carcinoma ($n=1$) and ductal carcinoma ($n=1$) (Fig. 2).

Pieces of fresh tumour tissue were used to set up short-term cultures from each neoplasm. The methods for tissue culture were described by Gibas *et al.* [18]. The mitotic activity and morphology of the cultured cells were assessed daily using a phase-contrast microscope.

Chromosomes were analysed using G-banding techniques and karyotypes were described according to the International System for Human Chromosomes Nomenclature (1991) [19].

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Received 6 Oct. 1995; accepted 20 Oct. 1995.

Table 1. *Summary of the clinical and cytogenetic results of* 19 *malignant salivary gland tumours*

Case	Age/sex	Classification	Karyotype
1	50/M	Mucoepidermoid ca	46, XY
2	12/F	Mucoepidermoid ca	46, XX
3	54/F	Mucoepidermoid ca	45, XX, −21[2]/47, XXX[2]/46, XX[9]
4	63/M	Mucoepidermoid ca	46, XY, t(2;7)(q23;p22)[5]/46, XY[6]
5	47/M	Mucoepidermoid ca	46, XY, +7, −21[2]/47, XY, +7[2]/94, XXYY, +7, +7[2]/46, XY, t(6;16)(q21;q22)[7]/46, XY, del(6)(q23)[1]/46, XY[3]
6	51/M	Acinic cell ca	45, X, −Y[3]/46, X, −Y, +7[1]/47, XY, +8[2]/46, XY[7]
7	41/F	Adenoid cystic ca	46, XX
8	46/F	Adenoid cystic ca	46, XX
9	32/M	Adenoid cystic ca	46, XY, t(6;9)(q23–25;p22–24)[8]/45, XY, t(6;9)(q23–25;p22–24), −20[4]
10	44/M	Epithelial–myoepithelial ca	46, XY
11	66/F	Epithelial–myoepithelial ca	48, XX, +2, +8[7]/45, X0, −X, −10, +20[2]/46, X0, −X, +20[1]/46, XX[6]
12	79/F	Myoepithelial ca	41, XX, −3, −7, −14, −17, −18[1]/43, XX, −8, −18, −19[1]/43, XX, −12, −18, −21[1]/46, XX[8]
13	31/F	Basal cell adenoca	46, XX
14	68/M	Ca ex-pleomorphic adenoma	46, XY
15	58/M	Ca ex-pleomorphic adenoma	46, X, −Y, +7[10]
16	72/F	Ca ex-pleomorphic adenoma	47, XX, +10[14]/46, XX[2]
17	65/M	Ductal carcinoma	46, XY, t(1;1)(p36;q12)[2]/47, XY, +7[1]/46, XY[9]
18	72/F	Adenocarcinoma NOS	47, XX, +7[1]/42, XX, −4, +7, −13, −15, −18, −20[1]/46, XX[4]
19	66/F	Undifferentiated ca	46, XX

ca, carcinoma; NOS, not otherwise specified.

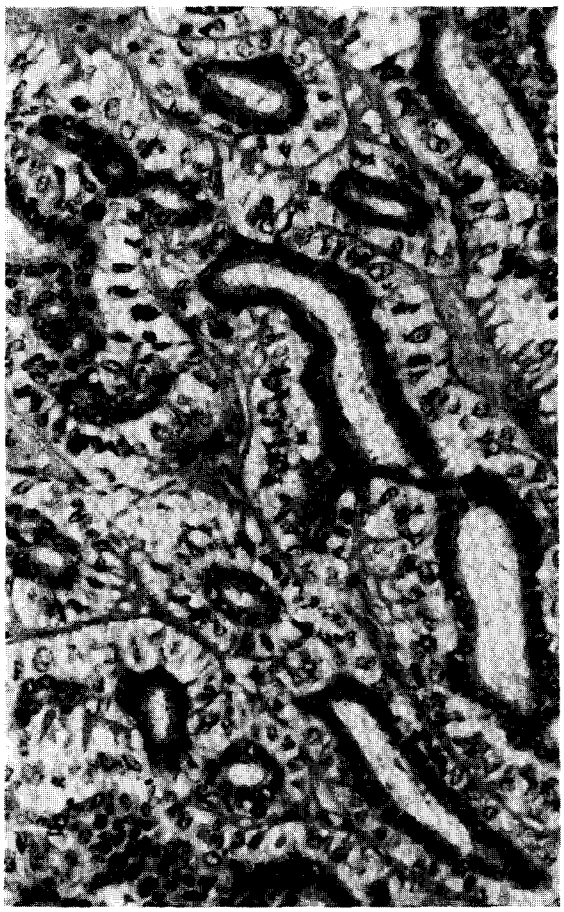

Fig. 1. Epithelial–myoepithelial carcinoma exhibiting a biphasic composition with an inner layer of dark, epithelial cells surrounded by a layer of clear, myoepithelial-type cells (case 11) (haematoxylin and eosin).

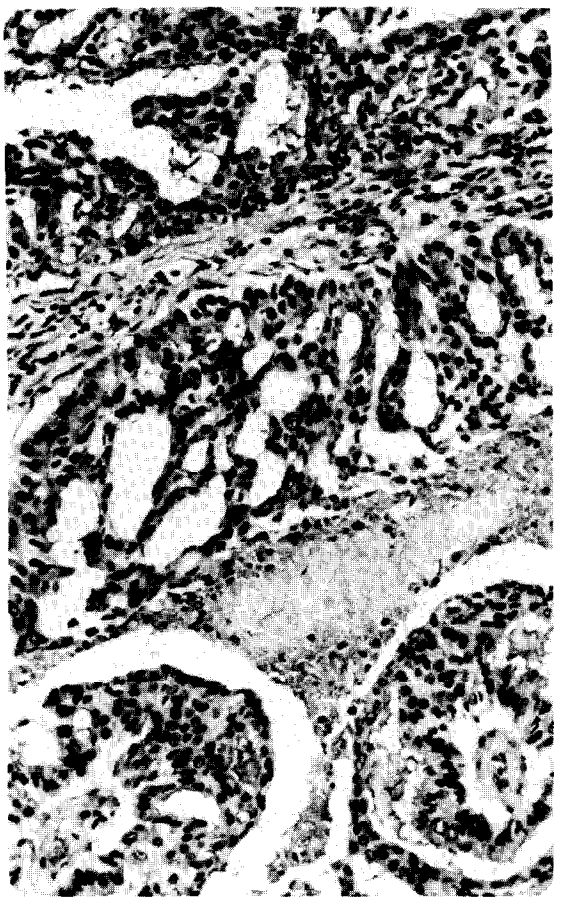

Fig. 2. Salivary duct carcinoma, showing a predominantly cribriform architecture (case 17) (haematoxylin and eosin).

The constitutional karyotypes were checked by routine lymphocyte cultures, except for cases 3, 4, 12, 14 and 19.

For DNA cytometric analysis, tumour samples were processed according to a modification of the method of Thornthwaite *et al.* [20]. Histograms were defined as diploid (DNA index = 1.0) if they had a single G0/G1 peak and as aneuploid if there was evidence of more than one distinct G0/G1 peak. Normal counterpart cells were used as a standard control. Cell cycle analysis was performed using the Multicycle Software Program (Phoenix Flow Systems, Inc., San Diego, California, U.S.A.).

RESULTS

Table 1 summarises the clinical data and the cytogenetic findings in 19 malignant salivary gland tumours. Flow cytometric DNA analysis was performed to compare the DNA index of the tumours with the modal chromosome number found by cytogenetic analysis in culture. The results allowed the acceptance of the cytogenetic analysis as representative of the original tumours. Flow cytometric DNA analysis revealed diploid histograms (DNA index = 1.0) for all tumours with the exception of cases 5, 14, 16 and 17 which exhibited a DNA index of 1.76, 1.24, 2.18 and 1.95, respectively.

All constitutional karyotypes analysed were cytogenetically normal. Eight of the 19 neoplasms had metaphases with normal karyotypes. They corresponded to two mucoepidermoid carcinomas, two adenoid cystic carcinomas, one epithelial–myoepithelial carcinoma, one carcinoma ex-pleomorphic adenoma, one basal cell adenocarcinoma and one undifferentiated carcinoma.

All the cases with cytogenetic abnormalities also displayed normal karyotypes, except cases 9 and 15. The former presented a t(6;9)(q23-25;p22-24) and the latter showed loss of chromosome Y and gain of chromosome 7 in all metaphases analysed.

Cases 3, 6, 11 (Fig. 3), 12, 15, 16 and 18 exhibited numerical alterations (gain of chromosomes 2, 7, 8, 10 and X and loss of chromosomes 18, 21 and Y), without concomitant structural anomalies. Cases 4 and 17 (Fig. 4) had reciprocal translocations as the sole abnormality, respectively, t(2;7)(q23;p22) and t(1;1)(p36;q12) and cases 5 and 9 showed both numerical and structural abnormalities with involvement of chromosomes 6, 7, 9, 16, 20 and 21.

DISCUSSION

Malignant tumours of the salivary glands are uncommon neoplasms. Their incidence is much lower than their benign counterpart and this fact may justify the rather limited karyotypic information available.

Our series of 19 malignant salivary gland neoplasms constitute a heterogeneous group of tumours, including ten different histopathological subtypes: mucoepidermoid carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, epithelial–myoepithelial carcinoma, myoepithelial carcinoma, basal cell adenocarcinoma, carcinoma ex-pleomorphic adenoma, ductal carcinoma, adenocarcinoma NOS and undifferentiated carcinoma. This histological diversity reflects the wide vari-

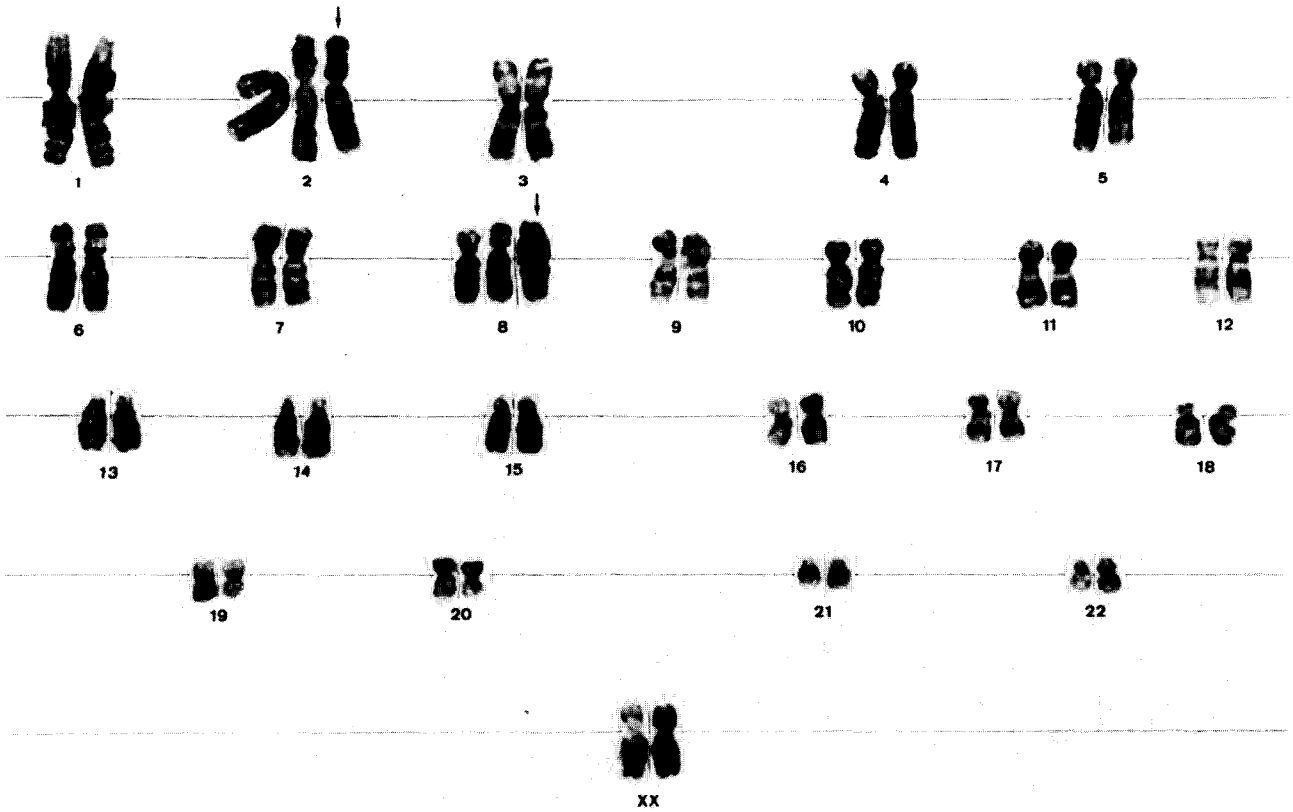

Fig. 3. Karyotype of a metaphase from case 11 (karyotypic description is shown in Table 1).

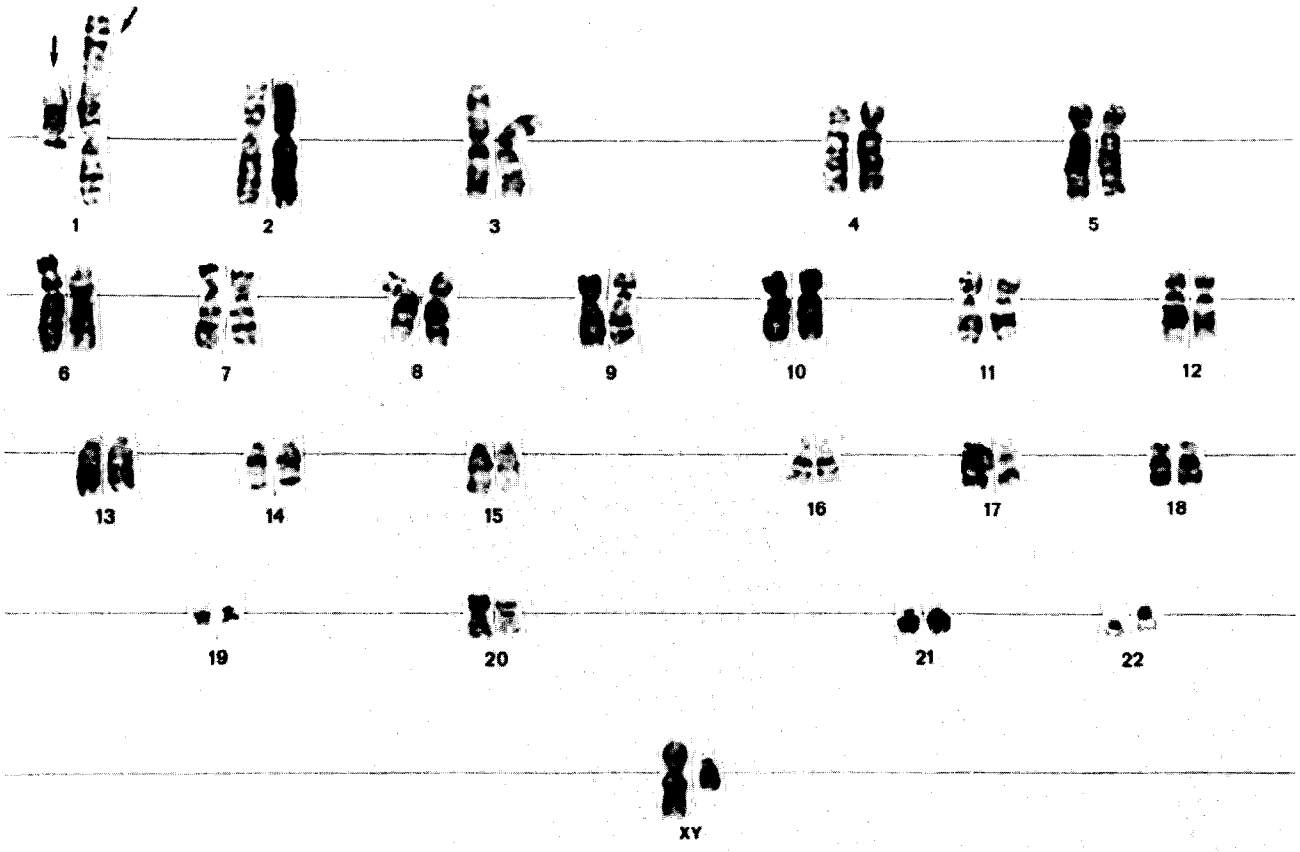

Fig. 4. Karyotype of a metaphase from case 17 (karyotypic description is shown in Table 1).

ation in the microscopical appearance of the malignant salivary gland tumours.

Flow cytometric results agreed well with the modal chromosome number found in cultured tumour tissue. For all tumours, except in cases 5, 14, 16 and 17, diploid histograms were in accordance with the modal chromosome number of the karyotypes. The aneuploid histograms obtained in cases 5, 14, 16 and 17 (1.76, 1.24, 2.18 and 1.95 DNA index, respectively), disagree with the modal chromosome number 46–47, found by cytogenetic analysis which can be explained by the loss of aneuploid cells in culture.

Eight cases revealed normal karyotypes and this finding is in part unexpected, considering the high frequency of clonal changes described in human malignancies [21, 22]. Due to the important role of the stromal mesenchymal cells in the composition of salivary neoplasms, one might argue that the apparently normal karyotypes are related to the high capacity of the stromal cells to grow *in vitro*. However, we can also admit that malignant neoplasms displaying normal karyotypes have alterations at a molecular level, without evidence of chromosomal rearrangements being, therefore, undetectable by conventional cytogenetical analysis.

Among salivary gland neoplasms, mucoepidermoid carcinoma is the best cytogenetically characterised subgroup with 18 cases already reported [11–15]. Numerical changes (loss of Y chromosome, trisomies as +7, +8) and structural abnormalities (6q22-25 and 11q14-24 rearrangements) are recurrent deviations found in these tumours. In our series, mucoepidermoid carcinoma was also the most frequent neoplasia ($n=5$). Three cases had clonal abnormalities: case 3, numerical deviations (loss of chromosome 21 and gain of chromosome X); case 4, a reciprocal translocation t(2;7)(q23;p22) and case 5, both numerical and structural deviations (loss of chromosome 21, gain of chromosome 7 and 6q rearrangements). Rearrangements of 11q14-24, namely the previously reported t(11;19)(q14-21;p12-13) [13–15] were not found in our cases.

Karyotypic information on acinic cell carcinoma includes some consistent clonal deviations: 6q rearrangements, loss of chromosome Y and gain of chromosomes 7 and 8 [14]. Apart from 6q rearrangements, case 6 also displayed trisomies 7, 8 and loss of chromosome Y. The pathogenic significance of −Y and +7 in short-term cultured neoplasms has been a matter of debate, since these aberrations were also found in non-neoplastic cells [14]. Recently, it has been demonstrated in kidney tumours and surrounding tissues that trisomies 7 and 10 characterise subpopulations of tumour-infiltrating lymphocytes [23]. The exact significance of these aberrations remains poorly understood. However, it is tempting to hypothesise a similar phenomenon occurring in the case we reported, since acinic cell carcinoma is a neoplasia characteristically containing aggregates of lymphoid cells.

The cytogenetic data of the 16 cases of adenoid cystic carcinoma recorded in the literature show 6q rearrangements, loss of chromosome Y and gain of chromosome 8 [4, 5, 14, 16]. One out of the three adenoid cystic carcinomas studied (case 9) revealed a reciprocal translocation t(6;9)(q23-25;p22-24) similar to the recurrent t(6;9)(q21-24;p13-23) found in three published cases of the parotid gland [4, 14, 17] as well as

to the t(6;9)(q23;p22) described in a case occurring at the lacrimal gland [15]. These findings indicate t(6;9) as a specific, and possibly primary non-random chromosomal abnormality in adenoid cystic carcinoma [14]. The other recurrent deviation already reported in adenoid cystic carcinoma, as well as in all major types of malignant salivary gland tumours, except in carcinomas ex-pleomorphic adenoma is the loss of genetic material from the long arm of chromosome 6, del(6)(q23-25) [4, 14, 16]. This aberration was not present in our series but the limited number of cases of this histological type do not allow definitive conclusions. However, we may admit that the normal karyotypes verified in two adenoid cystic carcinoma cases are due either to *in vitro* loss of neoplastic cell populations or to rearrangements at the molecular level, which are undetectable by chromosomal analysis.

Only 7 cases of adenocarcinomas of salivary glands were previously karyotyped, being identified loss of chromosome Y, gain of chromosome 8 and 6q deletions [4–7, 14]. The adenocarcinoma NOS of our series (case 18) only exhibited clonal numerical deviations represented by the gain of chromosome 7. Deletions of 6q, the most consistently recurrent aberration described in salivary gland adenocarcinomas, were not found in this particular case.

Case 15 displayed numerical deviations (−Y, +7) in all metaphases analysed and case 16 trisomy 10. To the best of our knowledge, only 6 cases of carcinoma ex-pleomorphic adenoma have been karyotyped: one case shared with one we described a trisomy 7, in addition to a 12q13-15 translocation [9, 10].

There is no cytogenetic information regarding salivary ductal carcinomas, basal cell adenocarcinomas, epithelial–myoepithelial carcinomas and myoepithelial carcinomas. In the present series, the malignant myoepithelioma (case 12) and one of the two epithelial–myoepithelial carcinomas (case 11) showed clonal numerical deviations. The former tumour displayed loss of chromosome 18 and the latter two unrelated clones: one had gain of chromosomes 2 and 8 and the other had loss of chromosomes 10, 20 and X. This cytogenetic biclonality may reflect the histologically biphasic composition of these low-grade adenocarcinomas. The fact that trisomy 8 was also detected in another carcinoma type suggests that chromosome 8 may be implicated in salivary gland tumorigenesis.

The salivary ductal carcinoma is a very rare and aggressive malignancy. The case studied showed the reciprocal translocation t(1;1)(p36;q12) and this is the first report on the involvement of this region in salivary carcinomas [24].

We conclude from our results and accumulated studies that the wide variation of cytogenetic findings may reflect heterogeneity in the histological types of malignant salivary gland neoplasms, a peculiar group of tumours known to have various morphogenetic pathways and diverse behaviour.

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Acknowledgement—This work was supported by a grant from the Comissão de Fomento da Investigação em Cuidados de Saúde do Ministério da Saúde, projecto 60/92.