Virchows Arch (1997) 430:339–342

CASE REPORT

Eva Röijer · Rigmor Dahlenfors · Joachim Mark
Göran Stenman

Observations by chromosome banding, FISH and immunohistochemistry in an adenoid cystic carcinoma with del(17)(p13) as the sole deviation

Received: 5 June 1996 / Accepted: 1 October 1996

Abstract We report on an adenoid cystic carcinoma (ACC) with a clonal deletion of 17p as the only karyotypic abnormality. Using different chromosome 17-derived probes we showed by FISH that the deletion encompassed the *p53* tumour suppressor gene. Immunohistochemical analysis revealed overexpression of *p53* protein in a subpopulation of cells, suggesting a mutation in the remaining *p53* allele in these cells. Our findings provide novel information about possible progressional pathways in ACC, and demonstrate the value of combining conventional cytogenetic analysis with molecular cytogenetic and immunohistochemical methods. This approach is particularly useful in cases with minor cytogenetic abnormalities at the border of visibility.

Key words Cytogenetics · FISH · p53 · Adenoid cystic carcinoma

Introduction

Adenoid cystic carcinoma (ACC) occurs primarily in the major and minor salivary glands. It is the fourth most common type of malignant salivary gland tumour [11] and occurs most often in adults during the fifth to seventh decades of life. It is a highly malignant neoplasm, with an often protracted clinical course. Late recurrences and metastases are common. The present report concerns the cytogenetic and molecular cytogenetic observations in an extremely slow-growing case of ACC originating from a minor salivary gland in the buccal mucosa. Cytogenetically, the tumour showed a 17p deletion as the sole anomaly. Using FISH and immunohistochemistry this anomaly was shown to involve the *p53* gene.

E. Röijer · G. Stenman (✉)
Laboratory of Cancer Genetics, Department of Pathology, Göteborg University, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden
Tel.: (46) 31-602922, Fax: (46) 31-820525

R. Dahlenfors · J. Mark
Department of Pathology and Cytogenetics, Central Hospital, Skövde, Sweden

Case report

A 43-year-old woman had been aware of a very slow-growing swelling in her right cheek for approximately 10 years. Clinical examination revealed a well-demarcated, firm, non-tender tumour, which was thought to be a pleomorphic adenoma. A complete local excision was performed. The specimen consisted of an unencapsulated, multinodular tumour measuring 0.5×0.5×0.5 cm, with solid, grey-white cut surfaces. Histological examination revealed a very highly differentiated ACC of the glandular type (Figs. 1a, b). An unusual feature was the lack of infiltrative growth.

The patient received postoperative X-ray treatment of local lymph nodes, and after 1 year there were no signs of local recurrence or metastases.

Materials and methods

Fresh tumour tissue was minced into small pieces and digested in a collagenase solution (1000 U collagenase/ml) for 30 min at room temperature. After washing twice in culture medium, the tumour cell suspension was seeded in 25 cm^2 flasks in Eagle's MEM supplemented with 10% fetal calf serum, 1% 200 mM L-glutamine, 200 units of penicillin/ml, and 50 µg of streptomycin/ml. Chromosome preparations were made from exponentially growing primary cultures. Cells were harvested after Colcemid exposure followed by hypotonic treatment and fixation in methanol:acetic acid. Slides were subsequently G-banded and analysed according to the recommendations of the ISCN [7].

FISH was performed on unbanded metaphase chromosomes using a human chromosome 17-specific painting probe (coatasome 17; Oncor, Gaithersburg, Md.), a *p53* cosmid probe (consisting of three partially overlapping cosmid clones covering 60–80 kb of genomic DNA; Oncor) and the alpha-satellite probe D17Z1 (Oncor). All probes were labelled with digoxigenin. The conditions for hybridization and post-hybridization washes were as recommended by the manufacturer. Immunohistochemical detection of the probes were performed using fluorescein isothiocyanate (FITC)-conjugated sheep anti-digoxigenin. Chromosomes were counterstained with propidium iodide. Slides were examined in a Zeiss Axiophot epifluorescence microscope using the appropriate filter combinations. Fluorescence signals were digitalized, enhanced and analysed using the ProbeMaster FISH image analysis system (Perceptive Scientific Instruments, Houston, Tex.). Colour

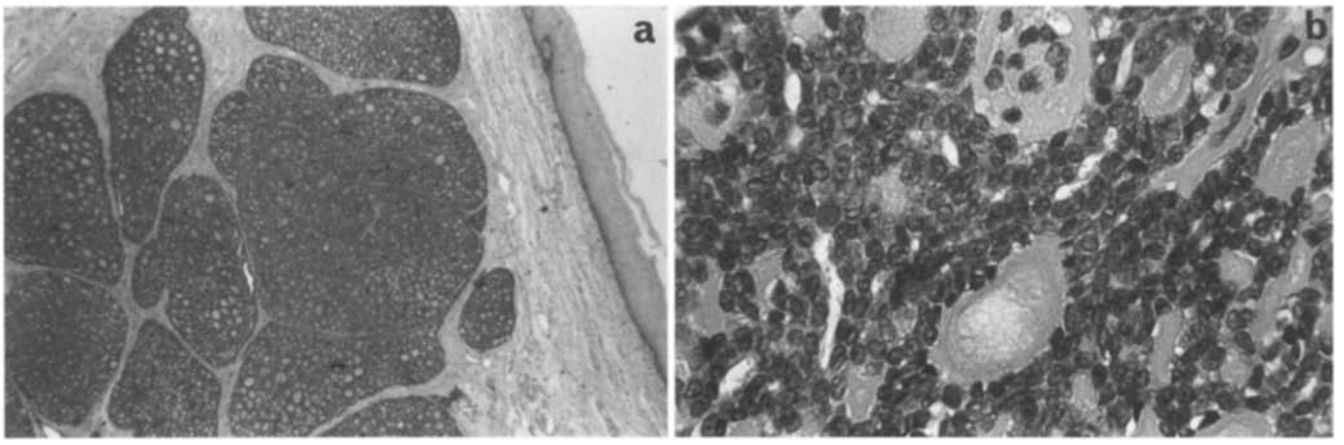

Fig. 1a Well-demarcated nodules of adenoid cystic carcinoma with no signs of infiltrative growth. H&E, ×25. **b** A tubular and cribriform growth pattern. H&E, ×100

prints were produced using a Kodak XL 7700 monochrome continuous printer.

Immunohistochemistry was performed on 4-μm sections from the formalin-fixed, paraffin-embedded tumour using the Dako TechMate 500 immunostainer (Dakopatts, Glostrup, Denmark). The *p53*-specific monoclonal antibody DO-7 (Dakopatts) recognizes an epitope in the N-terminus of the human *p53* protein corresponding to amino acid residues 35–45. Incubations with the primary antibody (1:500 dilution) were performed for 25 min at room temperature. Visualization of antibody binding was performed using the Dako ChemMate detection kit (Dakopatts). Sections were counterstained with haematoxylin. As a positive control, paraffin sections from a mucoepidermoid carcinoma with known *p53* mutation and *p53* immunoreactivity (unpublished data) was used.

Results

There was successful outgrowth from two cultures, harvested after growth periods of 8 and 10 days, respectively. A total of 42 metaphases were examined, 34 of which

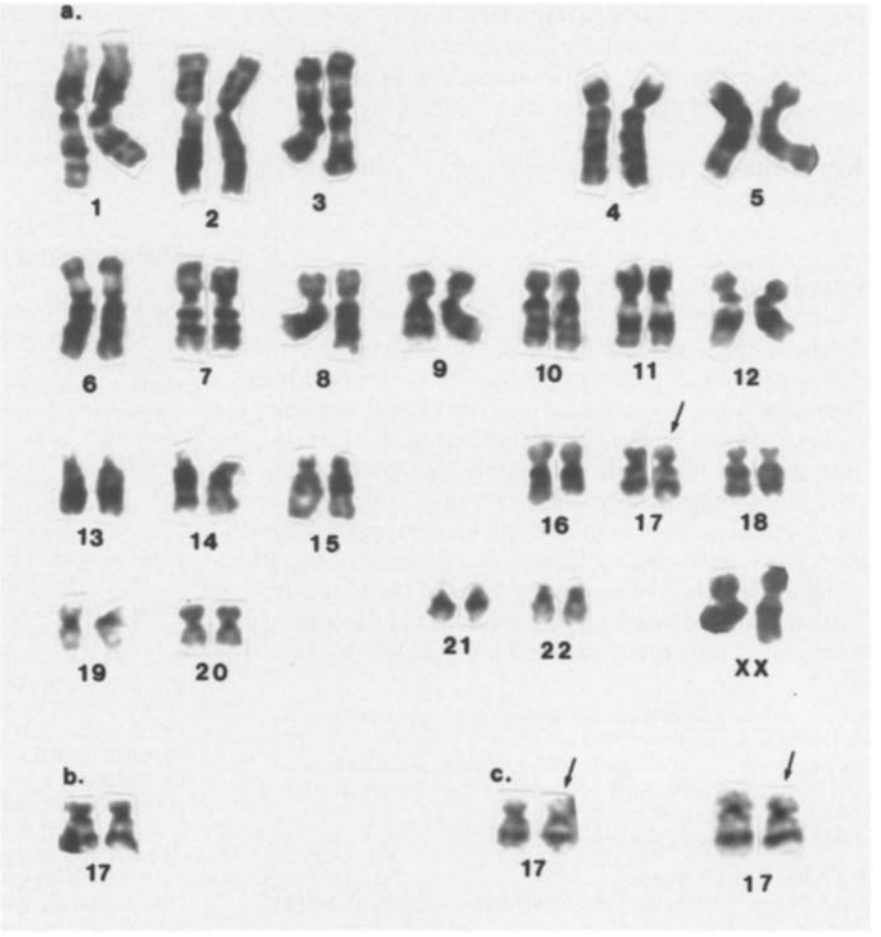

Fig. 2a Karyotype of a cell showing a minor 17p deletion (*arrow*) as the only deviation. **b, c** Partial karyotypes showing **b** two normal chromosomes 17 from one cell, and **c** short arm deletion of one chromosome 17 homologue in two cells (*arrows*)

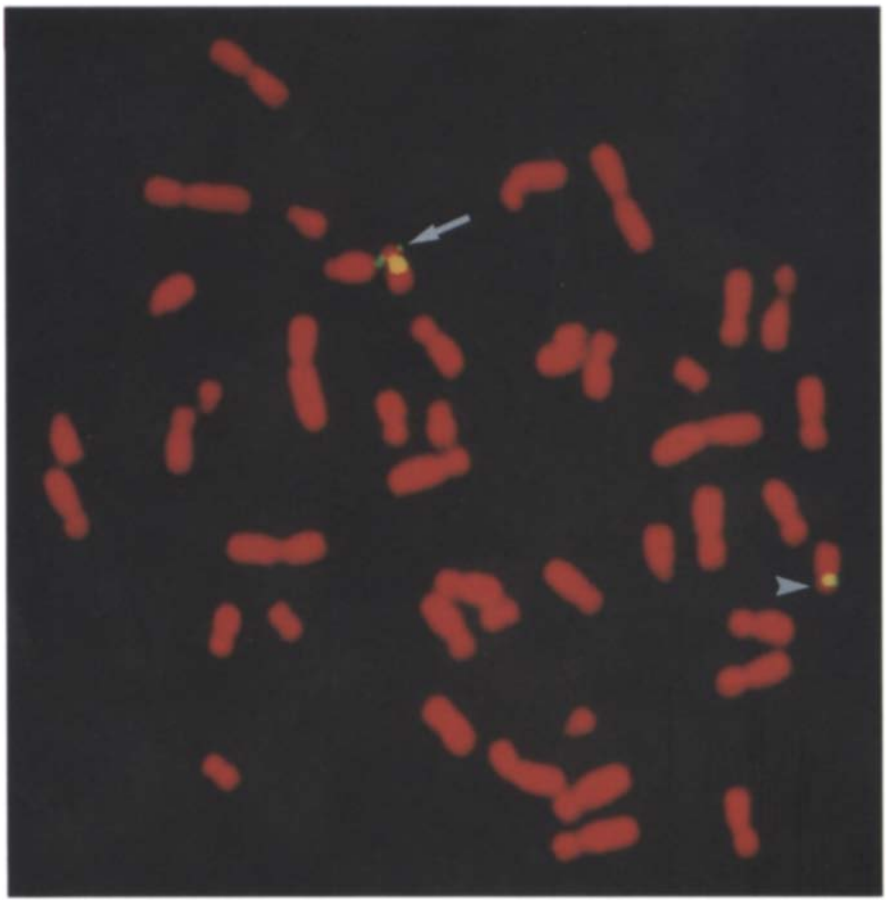

Fig. 3 Deletion mapping of the *p53* gene by FISH. Metaphases were co-hybridized with a chromosome 17-specific alpha-satellite probe and a *p53* cosmid probe. Note the presence of *p53*-specific hybridization signals on both chromatids of the short arm of one chromosome 17 (*arrow*) and the absence of signal from the other homologue (*arrowhead*). Chromosomes are counterstained in red with propidium iodide

had a perfectly normal, diploid karyotype and 1, a normal but doubled complement. The remaining 7 metaphases showed as the only deviation a small deletion of the short arm of one chromosome 17, i.e. del(17)(p13) (Fig. 2). Analysis of 15 metaphases from lymphocyte cultures from the patient revealed no difference between the p-arms of the two chromosomes 17.

Since the suggestive 17p deletion was at the border of visibility, we used FISH to characterize this rearrangement further. Hybridization with a chromosome 17-specific painting probe showed that there was no visible translocation of 17p material to any other chromosome in any of the 25 metaphases analysed. Since the *p53* gene maps to the region of 17p suspected to be deleted, we also co-hybridized a *p53*-specific cosmid probe and an alpha satellite probe for chromosome 17 to determine whether the deletion included the *p53* gene. Analysis of 25 hybridized metaphases showed that in 20% of the cells (5/25) one *p53* allele was lost (Fig. 3). In the remaining cells both copies of the *p53* gene were retained.

Immunohistochemical analysis revealed *p53* immunoreactivity in about 10–20% of the tumour cells. Focally, up to 50% of the cells were positive. The staining was strictly nuclear. Omission of the primary antibody from the incubations abolished the immunoreaction.

Discussion

In this communication we report on an ACC with a 17p deletion as the sole karyotypic anomaly. Using different chromosome 17-derived probes we demonstrated by FISH that the deletion encompassed the *p53* tumour suppressor gene. *p53* (also known as *TP53*), which encodes a nuclear phosphoprotein involved in the regulation of gene expression, cell cycle progression and apoptosis, is the most commonly mutated gene in human cancer [2, 5, 12]. Numerous studies have shown that *p53* mutations are often accompained by loss of the remaining wild-type allele. Immunohistochemical analysis of the present tumour revealed that 10–20% of the tumour cells stained positive for *p53* protein. The fact that one *p53* allele was lost in about 20% of the cells and that a similar fraction of cells showed accumulation of *p53* protein indicates functional inactivation of *p53* in a subpopulation of tumour cells. However whether the remaining *p53* allele is mutated or not is not known.

These observations are in agreement with recent studies of the *p53* expression and mutation patterns in a series of 300 benign and malignant salivary gland tumours, including 50 cases of ACC (A. Nordkvist et al., unpublished data). About 15% of the ACCs overexpressed (≥10% immunoreactive cells) the *p53* protein. Deletion and mutation analyses in two cases revealed loss of heterozygosity in one case, and loss of one allele and muta-

tion of the other allele in one case. These data together with the present observations indicate that *p53* is mutated and/or deleted in a subset of ACC.

The clinical and histopathological findings in the present case, together with the cytogenetic and molecular observations suggest that one or more submicroscopic mutations resulted in the actual development of this tumour and that the *p53* alterations were late events in the evolutionary chain. From this reasoning it follows that mutational changes at sites other than 17p13 are responsible for the development of the tumour. The other chromosome regions found to be preferentially affected in ACC (see below) are areas to be screened first for these critical initiating mutational changes [9].

Among the 29 previously published cases of ACC (references in [3, 4, 6, 8]), clonal changes were observed in 23 tumours. The following chromosome regions were preferentially affected: 6q13-24 (14 cases), 9p13-23 (8 cases), 17p12-13 (3 cases), Xp11.4–22 (3 cases) and 10p12-13 (2 cases). Of these deviations, those involving 6q, 9p and 17p included cases in which the rearrangements were obviously primary abnormalities. The most frequent and specific primary abnormality observed so far in ACC is a t(6;9)(q21-24;p13-23) [8]. This translocation is also diagnostic for ACC. The three previously reported ACCs with 17p involvement all had translocations with breakpoints in 17p12-13. One case showed, in addition to other clonal and non-clonal abnormalities, two separate clones with t(5;17)(q22;p13) and t(3;17)(p24;p13) respectively [10], and another showed a t(9;17)(p13;p13) as the sole anomaly [1]. The third case had a more complex karyotype with two related clones showing a t(17;18)(p12;q11.2) as the common aberration [8]. The present case thus represents the first ACC with a deletion involving 17p. Apart from providing novel information about possible progressional pathways in ACC this study clearly demonstrates the value of combining conventional cytogenetic analysis with molecular cytogenetic and immunohistochemical methods. This approach is particularly useful in cases with minor cytogenetic abnormalities at the border of visibility, and in cases where only a subpopulation of the tumour cells carry the rearrangement of interest.

Acknowledgements This study was supported by grants from the Swedish Cancer Society.

References

1. Higashi K, Jin Y, Johansson M, Heim S, Mandahl N, Biörklund A, Wennerberg J, Hambraeus G, Johansson L, Mitelman F (1991) Rearrangement of 9p13 as the primary chromosomal aberration in adenoid cystic carcinoma of the respiratory tract. Genes Chromosom Cancer 3:21–23
2. Hollstein M, Sidransky D, Vogelstein B, Harris CC (1991) *p53* mutations in human cancers. Science 253:49–53
3. Hrynchak M, White V, Berean K, Horsman D (1994) Cytogenetic findings in seven lacrimal gland neoplasms. Cancer Genet Cytogenet 75:133–138
4. Jin Y, Mertens F, Limon J, Mandahl N, Wennerberg J, Dictor M, Heim S, Mitelman F (1994) Characteristic karyotypic features in lacrimal and salivary gland carcinomas. Br J Cancer 70:42–47
5. Levine AJ, Momand J, Finley CA (1991) The *p53* tumour suppressor gene. Nature 351:453–456
6. López-Ginés C, Cérda-Nicolás M, Llombart-Bosch A (1994) Cytogenetic findings in a new case of adenoid cystic carcinoma arising in sphenoidal sinus. Cancer Genet Cytogenet 75: 150–152
7. Mitelman F (ed) (1991) ISCN Guidelines for cancer cytogenetics. (Supplement to an international system for human cytogenetic nomenclature) Karger, Basel
8. Nordkvist A, Mark J, Gustavsson H, Bang G, Stenman G (1994) Nonrandom chromosome rearrangements in adenoid cystic carcinoma of the salivary glands. Genes Chromosom Cancer 10:115–121
9. Sandros J, Stenman G, Mark J (1990) Cytogenetic and molecular observations in human and experimental salivary gland tumours. Cancer Genet Cytogenet 44:153–167
10. Stenman G, Dahlenfors R, Mark J, Sandberg N (1982) Adenoid cystic carcinoma: a third type of human salivary gland neoplasm characterized cytogenetically by reciprocal translocations. Anticancer Res 2:11–16
11. Tomich CE (1991) Adenoid cystic carcinoma. In: Ellis GL, Auclair PL, Gnepp DR (eds) Surgical pathology of the salivary glands. Saunders, Philadelphia, pp 333–349
12. Vogelstein B, Kinzler KW (1992) *p53* function and dysfunction. Cell 70:523–526