Pergamon

European Journal of Cancer, Vol. 35, No. 3, pp. 498–501, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(98)00368-2

Original Paper

Fluorescent *In Situ* Hybridisation (FISH) Characterisation of Pericentromeric Breakpoints on Chromosome 5 in Head and Neck Squamous Cell Carcinomas

C. Martins,[1] Y. Jin,[2] C. Jin,[2] J. Wennerberg,[3] M. Höglund[2] and F. Mertens[2]

[1]Department of Pathology, CIPM-Portuguese Cancer Institute, 1093 Lisbon, Portugal; [2]Department of Clinical Genetics; and [3]Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital, Lund, Sweden

Pericentromeric rearrangements, such as isochromosomes and whole-arm translocations, are frequently encountered in short-term cultures from head and neck squamous cell carcinomas (HNSCC). To characterise further the localisation of the breakpoints in such rearrangements, metaphase cells from seven HNSCC known to carry structural rearrangements of the pericentromeric region of chromosome 5 were investigated using fluorescent *in situ* hybridisation (FISH) techniques. With a whole chromosome painting probe it could be confirmed that all chromosome 5 rearrangements identified at cytogenetic analysis contained chromosome 5 material. By using a centromere-specific α satellite probe it could be shown, however, that cytogenetically identical derivative chromosomes had different breakpoints. Thus, we conclude that the results of the present investigation add further support to the hypothesis that the essential outcome of near-centromeric chromosome rearrangements is the creation of genomic imbalances, i.e. gain and/or loss of neoplasia-associated genes. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: head and neck, squamous cell carcinoma, cytogenetics, FISH, chromosome 5, centromere
Eur J Cancer, Vol. 35, No. 3, pp. 498–501, 1999

INTRODUCTION

CYTOGENETIC STUDIES of head and neck squamous cell carcinomas (HNSCC) have revealed a complex pattern of clearly non-random chromosome abnormalities [1–3]. The majority of observed structural chromosome aberrations result in genomic imbalances, the most frequent being loss of material from chromosome arms 3p, 7q, 8p, 13p, 14p and 15p and gain of 1q, 3q, 5p, 8q and 15q. Whereas a similar distribution of chromosomal gains and losses may be found in a variety of human malignancies [4], a peculiar feature of HNSCC is that they very often are caused by rearrangements that, at least at the cytogenetic level, seem to involve the centromeric regions of the chromosomes [2,5]. The majority of such rearrangements are isochromosome formations, notably i(1q), i(3q), i(5p), i(8q) and i(14q), and whole-arm translocations, but other types of structural aberrations with pericentromeric breakpoints have also been described [6]. The cause of the apparent clustering of breakpoints to centromeric or near-centromeric chromosome regions in HNSCC is unknown, but it could be noted that breakpoints detected in short-term cultures of non-neoplastic upper aerodigestive tract mucosa show the same type of distribution, and that the frequency of such rearrangements may be associated with smoking [7].

Although the most reasonable interpretation of the cytogenetic data is that the essential outcome of isochromosomes, whole arm translocations, and other alterations of pericentromeric DNA in HNSCC is the loss and/or gain of certain chromosome segments, one could not entirely dismiss the possibility that such rearrangements may lead to disruption of near-centromeric, cancer-associated genes. Support for this interpretation may be derived from fluorescent *in situ* hybridisation (FISH) studies of constitutional Xq isochromosomes, showing that often these do not originate from centromeric misdivision, but rather from unbalanced translocations with breakpoints in euchromatic segments on the short arm of the X chromosome [8]. More detailed analysis of centromeric rearrangements in HNSCC has so far been restricted to the demonstration that two whole-arm translocations, a

Correspondence to C. Martins, e-mail: carmomartins@mail.telepac.pt
Received 15 Apr. 1998; revised 4 Sep. 1998; accepted 10 Sep. 1998.

Table 1. *Fluorescent* in situ *hybridisation (FISH) analysis of chromosome* 5 *rearrangements in seven head and neck carcinomas*

Case no.	Age/sex	Site	Histology	Cytogenetic interpretation*	Revised interpretation after FISH
1	73/M	Floor of the mouth	SCC	der(5;22)(q10;q10), i(5)(p10)	
2	84/M	Laryngeal glottis	SCC	der(5;22)(q10;q10), ?i(5)(p10)×2	add(5)(p11)
3	64/M	Supraglottis	SCC	der(5)t(5;22)(p11;q11)	add(5)(p11)
4	77/M	Gingiva-bucca	SCC	del(5)(p11) der(5;17)(p10;q10)×2, i(5)(p10)×2	del(5)(p11p15)
5	56/M	Floor of the mouth	SCC	add(5)(q11)	
6	64/M	Retromolar trigone	SCC	der(3;5)(q10;p10)	der(3)t(3;5)(p11;p11)×3
7	49/M	Tonsil	Spindle cell carcinoma	der(5;17)(p10;q10), add(5)(q11)×2	der(17)t(5;17)(p11;p11)

SCC, squamous cell carcinoma; FISH, fluorescent *in situ* hybridisation. *The underlined rearrangements were revised after FISH.

der(X;11)(q10;q10) and a der(3;11)(q10; q10), both led to juxtapositioning of signals from centromere-specific α satellite DNA probes [5].

In the present study, we used FISH to analyse a selected series of seven HNSCC, all of which had been interpreted to have pericentromeric rearrangements of chromosome 5 at analysis with chromosome banding techniques.

MATERIALS AND METHODS

The present study was performed on metaphase cells from short-term cultures and established cell lines from seven primary HNSCC. Short-term cultures were obtained as described previously [1, 2]. All cases had been selected on the basis of their cytogenetic features, i.e. the presence of structural rearrangements involving the pericentromeric region of chromosome 5. In 3 cases, only short-term cultured cells were used for both cytogenetic and FISH analyses. In the other 4 cases, short-term cultures had been cytogenetically characterised, but the FISH analysis was performed on established cell lines. To minimise the risk of studying *in vitro* artefacts, the FISH analysis in these four cases included only those aberrations that had also been detected in short-term cultures. From each case, cells in fixative or on unstained slides had been preserved at − 20°C. Chromosome abnormalities were described according to ISCN [9].

FISH analysis was performed according to the method described by Höglund and colleagues [10]. The following probes were used: digoxigenin- or biotin-labelled centromere-specific probes for chromosomes 3 (cen3) and 17 (cen17), co-hybridising centromeric probes for chromosomes 14/22 (cen14/22) and 1/5/19 (cen1/5/19), a subtelomere-specific probe for the short arm of chromosome 5 (tel5p) (kindly provided by L. Kearney, Institute of Molecular Medicine, Oxford, U.K.) and whole chromosome painting probes for chromosomes 3 (wcp3), 5 (wcp5), 17 (wcp17) and 22 (wcp22). Cases 1 and 4 were analysed further using a pan-centromere probe (Cambio, U.K.). Hybridisations were analysed in a CytoVision ultra system (Applied Imaging, U.K.) using a charged coupled device (CCD) camera.

RESULTS

The chromosome 5 rearrangements identified at chromosome banding analysis and the description of these aberrations after FISH analysis are listed in Table 1. At banding

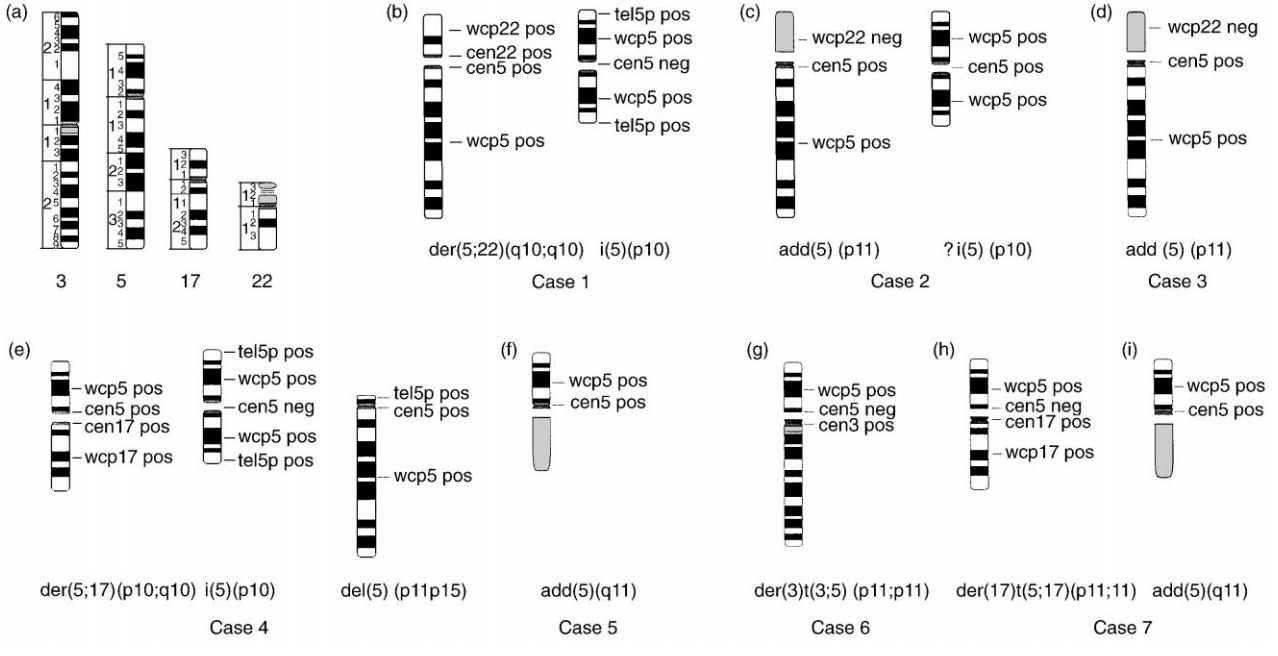

Figure 1. Schematic representation of fluorescent *in situ* hybridisation (FISH) results on chromosome 5 rearrangements in head and neck squamous cell carcinoma (HNSCC) cases (see Table 1). (a) Ideograms of chromosomes 3, 5, 17 and 22. (b-i) Cases 1–7: wcp, whole chromosome painting probe; cen, centromeric probe; tel, subtelomeric probe; pos, positive; neg, negative.

analysis, three recurrent aberrations were identified: i(5)(p10) (cases 1, 2 and 4), der(5;17)(p10;q10) (cases 4 and 7) and der(5;22)(q10;q10) (cases 1 and 2). In addition, case 3 had a der(5)t(5;22)(p11;q11), case 4 had a del(5)(p11), case 6 had a der(3;5)(q10;p10) and cases 5 and 7 had an add(5)(q11).

At FISH analysis with wcp5 it could be confirmed that all rearrangements involved chromosome 5 (Figure 1). By using additional centromeric and subtelomeric probes for chromosome 5 and centromeric and wcp probes for its translocation partners, we found that the aberrations were more heterogeneous than suspected from the banding analysis. Of the 3 cases with i(5)(p10) there were enough metaphase cells from two (cases 1 and 4) to confirm that they were isochromosomes and not deletions of 5q (Figure 2). With the probe for the centromere of chromosome 5, however, a positive signal was detected only in case 2, indicating that only a minor part of the centromeric α satellite DNA could have been included in the other two cases. α Satellite DNA sequences were detected when these 2 cases were analysed with a pan-centromere probe for centromeric sequences common to all chromosomes. In the two cases with a der(5;17)(p10;q10), one was positive for both centromeres, whereas the other (case 7) was positive for cen17 alone and should be described as

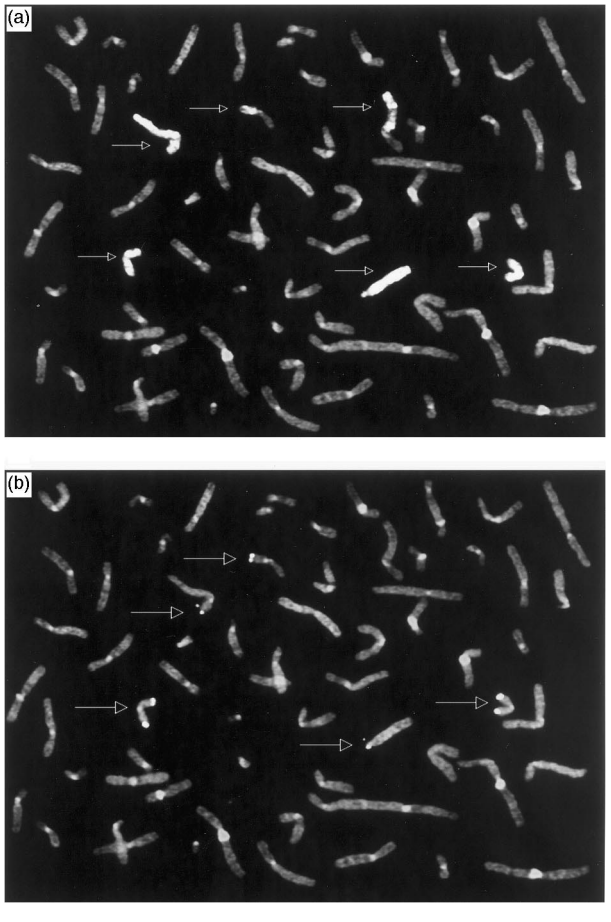

Figure 2. Fluorescent *in situ* hybridisation (FISH) analysis of case 4 with wcp5 probe (a) identifying a normal chromosome 5 (upper left arrow), a der(5;17)(p10;q10) (upper middle arrow), two i(5)(p10) (lower left and right arrows), and del(5)(p11p15) (lower middle arrow). All derivative chromosome 5 are indicated by arrows. (b) FISH analysis of the same metaphase cell using a subtelomeric 5p probe showing two signals on each of the suspected i(5)(p10) chromosomes.

der(17)t(5;17)(p11;p11). Of the 3 cases interpreted to have near-centromeric or whole-arm translocations between chromosomes 5 and 22, it turned out that only one, the der(5;22)(q10;q10) of case 1, could be verified by FISH. In the other two cases, the der(5) was negative for wcp 22 and, as there was not enough material to identify the translocation partners, the aberrations were re-interpreted as add(5)(p11). The der(3;5)(q10;p10) of case 6 was re-interpreted as der(3)t(3;5)(p11;p11) because it was positive only for the cen3 probe. The two rearrangements described as add(5)(q11) were both positive for the centromeric probe (cases 5 and 7), but there was not enough material to identify the translocation partners. Finally, the derivative chromosome 5 in case 4, described as del(5)(p11) at cytogenetic analysis, was positive for both the centromeric and the subtelomeric probes, thus, the aberration was re-interpreted as del(5)(p11p15) (Figure 2).

DISCUSSION

In the present study, seven HNSCC with near-centromeric rearrangements of chromosome 5 were analysed by FISH. The reasons for selecting cases with aberrations involving this particular chromosome were that i(5p) is one of the most frequent (10% of abnormal cases) structural rearrangements in HNSCC and that chromosome 5 has been repeatedly reported to participate in whole-arm rearrangements with other chromosomes as well [6].

The main conclusion that could be drawn from the FISH results is that although the cytogenetic results indicate a clustering of breakpoints within the centromeric region of chromosome 5 in HNSCC cells, a substantial variation in the localisation of the breaks can be found when the aberrations are subjected to more detailed analysis. Whereas all rearranged chromosomes containing the long arm of chromosome 5 yielded positive signals with the probe for the centromeric region, positive cen5 signals could only be demonstrated in four of eight derivative chromosomes containing 5p material, indicating that the α satellite DNA sequences detected by the probe used in this study extend further on the q arm than on the p arm and/or that these aberrations have more variable breakpoints. The difficulties in demonstrating α satellite DNA in 5p aberrations are well illustrated by the three cases with i(5)(p10). Whereas the chromosome 5-specific centromere probe gave signals in only one of the cases, the other two cases were shown to carry α satellite DNA sequences only by using a pan-centromere paint probe. The findings that one of the two tentative whole-arm translocations der(5;17)(p10;q10) and the suspected whole-arm translocation der(3;5)(q10;p10) were negative for the cen5 probe then does not necessarily exclude the possibility that they resulted from centromeric fusions.

The FISH results of the present study further support the hypothesis that the essential outcome of near-centromeric chromosome rearrangements in HNSCC is genomic imbalance, i.e. gain and/or loss of genes on 5p and 5q. Most of the cases (6 of 7) had over-representation of 5p, and only one tumour each showed a net loss of 5p (case 3) or 5q (case 7) material. Thus, it is reasonable to assume that 5p harbours a dominantly acting gene of importance in at least a subset of HNSCC.

1. Jin Y, Mertens F, Mandahl N, *et al.* Chromosome abnormalities in eighty-three head and neck squamous cell carcinomas:

influence of culture conditions on karyotypic pattern. *Cancer Res* 1993, **53**, 2140–2146.
2. Jin Y, Mertens F, Jin C, *et al.* Nonrandom chromosome abnormalities in short-term cultured primary squamous cell carcinomas of the head and neck. *Cancer Res* 1995, **55**, 3204–3210.
3. Van Dyke DL, Worsham MJ, Benninger MS. Recurrent cytogenetic abnormalities in squamous cell carcinomas of the head and neck region. *Genes Chromosomes Cancer* 1994, **9**, 192–206.
4. Mertens F, Johansson B, Höglund M, Mitelman F. Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. *Cancer Res* 1997, **57**, 2765–2780.
5. Hermsen MAJA, Joenje H, Arwert F, *et al.* Centromeric breakage as a major cause of cytogenetic abnormalities in oral squamous cell carcinoma. *Genes Chromosomes Cancer* 1996, **15**, 1–9.
6. Mitelman, F. *Catalog of Chromosome Aberrations in Cancer*, 6th edn. New York, Wiley-Liss (in press).
7. Jin C, Jin Y, Wennerberg J, *et al.* Clonal chromosome aberrations accumulate with age in upper aerodigestive tract mucosa. *Mutat Res* 1997, **374**, 63–72.
8. Wolff DJ, Miller AP, Van Dyke DL, Schwartz S, Willard HF. Molecular definition of breakpoints associated with human Xq isochromosomes: implications for mechanisms of formation. *Am J Hum Genet* 1996, **58**, 154–160.
9. ISCN. *An International System for Human Cytogenetic Nomenclature*. Mitelman, F., ed. Basel, Karger, 1995.
10. Höglund M, Johansson B, Pedersen-Bjergaard J, Marynen P, Mitelman F. Molecular characterization of 12p abnormalities in hematologic malignancies: deletion of *KIP1*, rearrangement of TEL, and amplification of *CCND2*. *Blood* 1996, **87**, 324–330.

Acknowledgements—This study was supported by grants from the Swedish Cancer Society, the Smokeless Tobacco Research Council and the Inga Britt and Arne Lundberg Foundation. Carmo Martin's visit to Lund, Sweden was supported by a grant from the Swedish Institute.