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Centromeric chromosomal translocations show tissue-specific differences between squamous cell carcinomas and adenocarcinomas

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Structural chromosomal aberrations are common in epithelial tumors. Here, we compared the location of centromeric breaks associated with whole arm translocations in seven adenocarcinoma cell lines and nine squamous cell carcinoma cell lines using SKY, microarray-based comparative genomic hybridization (array CGH) and fluorescence *in situ* hybridization (FISH). Whole arm translocations were more frequent in squamous cell carcinomas (112 in nine cell lines and nine in one short-term culture) than in adenocarcinomas (13 in seven cases) and most often resulted in copy number alterations. Array CGH analysis demonstrated that in all squamous cell carcinomas and in most adenocarcinomas, the breakpoints of unbalanced whole arm translocations occurred between the two clones on the array flanking the centromeres. However, FISH with centromeric probes revealed that in squamous cell carcinomas, the marker chromosomes with whole arm translocations contained centromeres comprised of material from both participating chromosomes, while in adenocarcinomas centromeric material from only one of the chromosomes was present. These observations suggest that different mechanisms of centromeric instability underlie the formation of chromosomal aberrations in adenocarcinomas and squamous cell carcinomas.
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Introduction

Epithelial cancers are the most frequent type of malignant tumors, and they have a large impact on human morbidity and mortality. Recurrent and stage-specific chromosomal aberrations have been observed in many tumor types (NCI and NCBI's SKY/M-FISH and CGH Database, 2001; Albertson *et al*., 2003). How these chromosomal changes affect basic cellular mechanisms and drive cell transformation and tumor progression at the molecular level has only been resolved to a limited extent. In addition, the mechanisms leading to tumor-specific numerical and structural chromosomal aberrations are not well understood.

It has been recognized for some time, however, that tumors arising in individuals with mutations in genes that function in particular pathways that maintain genomic integrity such as *BRCA1* (Tirkkonen *et al*., 1997; Israeli *et al*., 2003) or DNA mismatch repair (Schlegel *et al*., 1995; Eshelman *et al*., 1998; Soulie *et al*., 1999; Nakao *et al*., 2004) are associated with particular patterns of chromosomal aberrations. Analyses of a large series of tumor samples using comparative genomic hybridization (CGH) have revealed not only characteristic recurrent chromosomal gains and losses in adenocarcinomas (malignant tumors arising from glandular epithelium) compared to squamous cell carcinomas (malignant tumors arising from squamous epithelium) from different sites of origin (Hermsen *et al*., 1998, 2001, 2002; Meijer *et al*., 1998; Jin C *et al*., 2000; Grieken *et al*., 2000), but also a higher frequency of centromeric breakage and imbalances involving whole chromosome arms in squamous cell carcinomas compared to adenocarcinomas (Hermsen *et al*., 1996; Petersen *et al*., 1997; Luk *et al*., 2001; Pei *et al*., 2001; Varis *et al*., 2001; Weiss *et al*., 2003).

To investigate these differences in more detail, particularly with regard to the structural abnormalities involving the centromere, we performed a comprehensive analysis of chromosomal aberrations in two series of established adenocarcinoma (colorectal) and squamous cell carcinoma (oral cavity) cell lines using spectral karyotyping (SKY), array CGH and fluorescence *in situ* hybridization (FISH). This comprehensive approach

revealed differences in the formation of centromeric translocations in adenocarcinomas and squamous cell carcinomas. Notably, in squamous cell carcinomas the centromeres of marker chromosomes resulting from translocations involving whole chromosome arms were composed of material from both of the participating chromosomes, whereas in adenomas centromeric material of only one chromosome was present, suggesting that different defects in the maintenance of centromeric stability underlie the formation of structural chromosomal aberrations in these two tumor types.

Results

Cytogenetic characterization of cell lines

In all cell lines, numerical and structural chromosomal aberrations were found by SKY. In adenocarcinoma cell lines, on average 11.1 (range 2–27) chromosomes with structural and/or numerical abnormalities were clonally present compared to 19.4 (range 3–28) in the squamous cell carcinoma cell lines. In addition, on average 5.6 (range 0–12) nonclonal structural aberrant chromosomes (i.e. found in one cell only) were identified in the adenocarcinoma cell lines compared to 17.2 (range 0–33) in the squamous cell carcinoma cell lines, respectively. A full description of all karyotypes is presented in Table 1. SKY analysis also revealed that two adenocarcinoma cell lines, WiDr and Colo205, were derivative of HT29, although they had originally been established as unique tumor cell lines from three different patients, confirming a previously reported observation for WiDr and HT29 (Chen *et al*., 1987). These three cell lines had the majority of structural abnormalities in common, although a number of markers occurred in only one or two of the three. For our analysis of different types of chromosomal aberrations (see below), we therefore considered them as representing one cell line, leaving seven unique adenocarcinoma cell lines in the study.

In both types of cell lines, adenocarcinoma and squamous cell carcinoma, the following types of rearrangements were found besides numerical aberrations: unbalanced translocations, whole arm translocations, deletions, duplications, isochromosomes, complex translocations and homogeneously staining regions. Reciprocal translocations, including inversions, occurred in all adenocarcinoma cell lines, but only once in a single squamous cell carcinoma cell line. Karyotype examples of a colorectal adenocarcinoma and an oral squamous cell carcinoma are shown in Figure 1a and b.

Recurrent structural aberrations in the adenocarcinomas included isochromosomes i(3)(q10), i(8)(q10), i(15)(q10) and i(20)(q10). In the squamous cell carcinomas, the following isochromosomes and whole chromosome arm translocations were observed in two or more cases: i(3)(q10), i(5)(p10), i(8)(q10), i(10)(q10), i(18)(p10), i(20)(q10), i(21)(q10), i(22)(q10), and der(1;10)(q10;q10), der(1;18)(q10;p10), der(2;11)(p10;p10), der(4;12)(p10;p10), der(5;18)(p10;p10), der(5;19)(p10;p10), der(6;14)(p10;q10), der(11;22)(q10;q10), and der(15;18)(q10;p10). Recurrent translocations involving defined chromosome bands were found in neither of the two tumor types.

Figure 1c shows the frequency distribution of whole chromosome arm involvement in translocations in squamous cell carcinomas and adenocarcinomas. There is a clear difference in overall frequency as well as in participation of specific arms. In squamous cell carcinomas, centromeres of all chromosomes were involved, while in adenocarcinomas this was only the case for 17 of 24 chromosomes. Moreover, in all squamous cell carcinomas, some chromosome arms were involved in two or more whole arm translocations with different partners. For example, in case scc1131, chromosome 22 was translocated to 1q and 10q, while in scc120 chromosome arm, 18p was involved in four different translocations (Figure 2a) and 12p in six (data not shown).

The comparison of array CGH and SKY analysis of these cell lines indicated that copy number transition points (i.e. loci with a significant change in fluorescence ratio, Snijders *et al*, 2003) were consistent with the breakpoints of structural rearrangements detected by SKY. However, array CGH provided a higher resolution mapping of the position of the breakpoint to an average resolution of 1.4 Mb. For unbalanced whole chromosome arm translocations both in squamous cell carcinomas and adenocarcinomas, we were able to locate the breakpoints in between the two BAC clones flanking the centromeres (Figure 2b). Whole arm translocations without accompanying gain and/or loss were observed in a number of cases, but only in squamous cell carcinomas.

Classification of chromosome rearrangements

In order to categorize different types of chromosomal rearrangements, based on a combined interpretation of SKY and array CGH, we defined three classes: (1) band–band rearrangements, in which the breakpoints of both partner chromosomes are located on the chromosome arms; (2) band–centromere rearrangements, in which one of the partner chromosomes is broken and fused within the arm and the other partner within the centromere; and (3) centromere–centromere rearrangements, in which both partner chromosomes are broken and fused within the centromere, leading to isochromosomes and whole arm translocations. Only clonal aberrations were considered in this analysis. Band–band rearrangements were observed in 54% of the adenocarcinomas, as compared to 31% of the squamous cell carcinomas, whereas centromere–centromere rearrangements occurred more frequently in squamous cell carcinomas than in adenocarcinomas, 58 and 25%, respectively. Band–centromere rearrangements occurred with comparable frequency in both adenocarcinomas and squamous cell carcinomas, 21 *vs* 11%, respectively (Table 2).

Table 1 Composite karyotypes of nine cell lines and one short-term culture of squamous cell carcinoma and of nine colon cancer cell lines, based on SKY, CGH and array CGH data

scc040	46,X-X,dup(3)(q22.2-qter),+9,der(18)t(8;18)(q21.13;q23) [14] /46,X-X,dup(3)(q22.2-qter) +9,der(12)t(X;12)(q21.3;q24.33),der(18)t(8;18)(q21.13;q23) [5]
scc059	58–70,<3n>,X,−X,der(X;3)(q10;p10),+der(1;5)(p10;q10),der(2;5)(p10;p10), der(2;18)(q10;p10),+i(2)(p10),+der(3)t(3;8)(p11;q?), +der(3;16)(q10;q10),−4,+5, +der(5;13)(p10;q10),+i(5)(p10),−6,−8,der(8)t(8;8)(q?;q?),−9, der(10)t(9;10)(q21.11;q21.2),−11,hsr(11)(q13),der(12)t(10;12)(p?;q22), +i(15)(q10), +16, der(17;20)(q10;p10),der(17)t(12;17)(p11;q21.32)hsr(17)(q21.32),+18,+20, +der(20)t(7;20)(?;q11.2),−21,−21, der(21)t(8;21)(q22.2;p11),−22,der(22)t(14;22)(q12;q12), i(22)q10) [cp 9]
scc078	63–66,<3n>,der(X;11)(q10;q10)x2,(Xqter-q10::hsr9q34::3q10-qter),+i(X)(q10),der(1;18)(q10;p10), dup(1)(q10q12),der(3)t(3;?) (p25;?),+i(3)(q10),der(4;12)(p10;p10), der(5;18)(p10;p10),der(6)t(6;17)(q25.3;q21.33),der(7;17)(p10;q10)t(8;17)(q21.11;q21)t(8;17) (q21.13;q21)t(8;17)(q21.11;q21)t(7;8)(p11;q21.13), +der(7)t(7;17)(q11;?)t(17;18)(?;p11),+(7pter-p10::8q10–q12.1::17p11–p12::8q12.1–q10::7p10-pter),+der(8;17)(q10;q10)del(17)(q21),+i(8)(q10),−9, t(10;15)(q23.31;q14)x2,der(11)t(1;11)(p21.3;q14),+der(11) t(9;11)(p13;q14),+12, der(13;14)(q10;q10),+14,der(15)t(3;15)(p21;q14)dup(15)(q12q14),−16,+17,−19,+20, dup(21)(p11.1q11.1), i(21)(q10) [cp 13]
scc080	58–78,<3n>,X,X,der(Y;1)(q10;q10),+der(Y;1)(q10;q10)del(1)(q31),+der(1;15)(p10;q10),+der(1;15)(p10;q10)t(1;15)(q42;q26.3), +der(2;20)(p10;q10),i(3)(q10), der(4)t(3;4)q24;q33),del(5)(q11),der(5;18)(p10;p10),+der(6;11)(q10;q10),del(7)(q31.1),+der(7)t(7;10) (q31.1;q26.11),−8,der(8;10)(q10;p10),−9,der(9;18)(q10;p10),−10, i(10)(q10),+der(11;22)(q10;q10)hsr(11)(q13),del(12)(q13),der(12) t(2;12)(q11;q14.3),+i(12)(p10),−13,der(14;18)(q10;q10), der(14)t(12;14)(q11;p11),+15, ider(15)(q10)del(15)(q14),+der(16)t(16;17) (p11;p12)hsr(17)(p11.2p12)t(5:17)(q13;p12),+der(17)t(5;17)(q13;p12)hsr(17)(p11.2p12),+18,+18,+i(18)(p10), der(19)t(12;19) (q13;p13.2),+20,i(21)(q10) [cp 10]
scc094	31–78,<3n>,der(X)(X;X)(p?;p?)x2,i(X)(q10),der(1;14)(p10;q10),i(1)(q10),+2,i(3)(q10),+i(4)(q10),+5,+del(7)(q32),der(8;8) (q10;q10)t(6;8)(?;q24.3),+i(8)(q10)x2, del(9)(p21p21),+del(9)(p21p21),+i(9)(q10),dic(13;22)(p11;p11)x2,i(13)(q10), del(15)(q21q22),dic(15;20)t(p11;q13.3)del(15)(q21q22),+16,+del(17)(p12),−18,+19,−21, der(21;22)(q10;q10),i(21)(q10),−22,der(22)t(1;22)(p21.3;p11) [cp 12]
scc096a	34–62<3n>,X,−X,Y,der(1;9)(q10;q10),der(1;11)(q10;p10),+der(1;11)(p10;q10)t(11;17)(q23.1;q12),der(2)t(2;12)(q34;p12), +der(2;22)(q10;q10)t(2;22)(q37;q13), del(3)(p13p21),−4,der(4)t(4;4)(q32.1;q28.3),der(7;10)(q10;q10),+der(7;13)(p10;q10)x2,−8, der(8)t(8;8)(p21.2;q21.11),−9,+der(11)t(9;11)(q12;q13)hsr(11)(q13), der(12)t(3;12)(q25;q15),+der(12)t(12;14)(q13;q11),−13,−14,−14, der(14)t(1;14)(q32.1;p11)t(1;3)(q44;q11),der(15;18)(q10;p10),−17, der(18)t(9;18)(q21;p11)t(7;18)(p15;q23),+der(18)t(12;18) (q12;q12.3),der(19;21)(q10;q10),der(19)t(1;19)(q32.2;p11),der(20)t(4;20)(q25;q13.3),−21,i(21)(q10), +22,+del(22)(q11.2),+der(22) (X,22)(q25;q11.2),+i(22)(q10) [cp 10]
scc120	55–83,<4n>,X,X, −X,der(X;3)(q10;q10),del(1)(q12),der(1;10)(q10;q10)x2,der(1;2)(p10;q10),+der(1)t(1;3)(p36.3;q24),−3,der(4;12) (p10;p10),der(5;14)(q10;q10), i(5)(p10),+i(5)(p10),−7,der(7)t(7;11)(q11.1;q14.1)t(11;19)(q22.1;q13.3),−8,+9,+9,−10,−11, der(11;12)(p10;p10),+der(12;18)(p10;p10),der(13)t(12;13)(q23.1;p11)x2,−14,−15,−15,−16,der(16)t(12;16) (q21.31;p13.3)t(3;12)(q24;q24.3),del(18)(q12),+der(18;20)(p10;p10),+der(18)t(1;18)(p31.1;q11)t(X;1) (q25;p34.3),−19,−19, der(19)t(15;19)(q11;p12),+20 [cp 6]
scc147	69–71,<3n>,X,X,−Y,der(2)t(2;5)(q21.2;p13.3),ider(2)(q10)del(2)(q33.2q37.1),−3,der(3)t(3;3)(p21;q24)t(3;15)(q29;q22),−4, der(4;17)(p10;p10), der(4)t(3;4)(q25;p16)dup(3)(q24q29)t(3;3)(q29;p21.32),+der(5;20)(p10;p10),der(6;14)(p10;q10),+der(6;14) (p10;q10),del(7)(q22),+del(7)(q32), der(7)t(3;7)(q24;q32)t(3;13)(q26;q22),−8,+9,der(10)t(3;10)(p21;q11.22),del(11)(q13) hsr(11)(q13),+del(11)(q13)hsr(11)(q13),−13,+14, der(15)t(9;15)(q31.3;p11)t(8;15)(q22.2;q15),+16,del(17)(p11.2),der(17;19) (p10;q10),+der(17)t(17;18)(p12;q11.22)del(17)(q22),−18,+del(19)(p11),del(20)(p12.1), +i(20)(q10),+dmin11 [cp 10]
scc1131	71–74,<3n>,X,X,X,+del(X)(p11),+der(X)t(X;22)(q11;q11)t(1;22)(?;q13),der(1;10)(q10;q10),+der(1;14)(q10;q10),+der(1;22) (q10;q10),der(3)t(3;14)(p25;q23.3), +i(3)(q10),−4,+i(5)(p10),der(6;12)(p10;p10),+i(7)(p10),del(8)(p12),der(8)t(8;22)(p23;q12), +i(8)(q10),+del(9)(p23),del(10)(p11.1),+der(10)t(9;10)(q31;q26), +der(11;13)(p10;q10), +der(11;22)(q10;q10),+del(12)(p11) del(12)(q22),+der(13;13)(p11;q14),tas(14;20)(p13;q13.3),der(17)t(6;17)(q24;p12), +der(17)t(6;17)(q25.2;p12),der(18)t(1;18)(p21.2;q23) x2,der(18)t(1;18)(p31;q23)t(X;1)(p11.1;p32),+der(18)t(3;18)(p14;q11),+i(18)(p10),+del(20)(p11),+21,+22,+22, +der(22) t(13;22)(q14;q12) [cp 18]
scc1365	58–82<4n>,XX,−Y,−Y,der(2;8)(p10;p10),2xder(3;21)(q10;p10),2x der(4)t(4;13)(p14;q13),+2xder(4;8)(p10;q10),+der(4;6)(q10;p10), 2xdel(5)(q12)amp(5)q11.2), +der(6)t(6;8)(q21;q?),+8,der(8;14)(q10;q10),2xder(8;14)(q10;q10)t(X;8)(q25;q22),2x der(9;13)(q10;q10), −10,2xder(11)t(11;12)(q22;p12)amp(11)(q22), 2xder(12;16)(q10;q10),+del(12)(p11),−13,−13,−14,−14,−14,i(14)(q10),−15, der(15;21)(q10;q10),−18,2xder(18)t(15;18)(q23;q23),del(19)(p12),+20,del(20)(p13),−21,−21, 2xder(21)t(8;21)(q22;p11),−22,del(22) (q12) [cp 13] /136–158,<4n>,idemx2 [cp 3]
HT29	65−71,<3n>,X,X,del(X)(p11.3),der(3)t(X;3)(p21.2;q26.33)t(3;3)(p11;q11.2–q22.2),ins(3;12)(p11;p11–p12),der(4)t(4;21)(q31;q11.1), +der(5)t(5;6)(q11;?), del(6)(q12)dup(6)(q12),t(6;14)(q23;q13),+t(3;6;14)(q21;q23;q13)ins(3;12)(p11;p11.p12),+del(7)(p21),der(8) t(8;8)(q10;q10)dup(8)(q23.2q24.3), +der(8)t(8;8)(q10;q10)t(8;10)(?;p15)dup(8)(q23.2q24.3),ins(9;X)(q22;?),+der(10)t(8;10)(?;p15), +11,−13,der(13)t(13;13)(p11;q12.3),i(13)(q10),−14,+15, der(17;19)(q10;q10),i(18)(p10),+del(19)(p13.2),+der(19)t(19;19) (p13.3;q13.1),+20,+i(20)(q10),−21,−21,−22,+der(?)t(?;17)(?;q11.2)del(17)(q21.2) [cp 21]
WiDr	67–69,<3n>,X,X,del(X)(p11.3),+der(X;17)(q10;q10)t(6;17)(?;?),der(1)t(1;2)(q23.3;?),der(2)t(2;2)(q33;p16.1),der(3)t(X;3) (p21.2;q26.33)t(3;3)(p11;q11.2–q22.2), +ins(3;12)(p11;p11–p12),del(4)(q31),der(5)t(5;6)(q11;?),+der(5)t(5;13)(p15.33;q13.1),6, t(6;14)(q23;q13),del(7)(p21),der(8)t(8;8)(q10;q10)dup(8)(q23.2q24.3), +ins(9;X)(q22;?),+11,−13, i(13)(q10),−14,+15,+der(17;19) (q10;q10),der(18)t(7;18)(p21;q23),i(18)(p10),+20,−21,−22,+der(?)t(?;17)(?;q11.2)del(17)(q21) [cp 13]
Colo205	67–72,<3n>,X,X,del(X)(p11.3),der(3)t(X;3)(p21.2;q26.33)t(3;3)(p11;q11.2–q22.2),+ins(3;12)(p11;p11–p12),del(4)(q31),der(5) t(5;6)(q11;?), +der(5)t(5;13)(p15.33;q13.1),der(6)t(5;6)(p15.33;p21.33),t(6;14)(q23;q13),del(7)(p21),der(8)t(8;8)(q10;q10)dup(8) (q23.2q24.3),ins(9;X)(q22;?),+11,−13,i(13)(q10),14, +15,+der(17;19)(q10;q10),der(18)t(7;18)(p21;q23),+i(18)(p10),+19,+20, −21,−22,+der(?)t(?;X)(?;?),+der(?)t(?;17)(?;q11.2)del(17)(q21) [cp 6]
Colo320	50–55,<2n>,der(X;10)(q10;q10),t(1;5)(p10;q10),t(1;16)(p36.1;p11.1),+der(1)t(1;14)(p12;q11)t(1;13)(q24.2;q11),der(2)t(2;10) (q11;?),der(2)t(2;?)(p11;?), +ider(2)(p10)ins(2;8)(p16;q24.1),der(3)t(3;9)(q12;p21),der(4)t(4;7)(q27;?),+der(4)t(4;9)(p15.33;q34.13) t(3;4)(q12;q25)t(3;16)(q27;q22), der(5)t(1;5)(p12;p11)del(1)(p13),+der(5)t(1;5)(p12;p11)t(1;8)(p21.2;?)t(3;8)(q22.3;?),t(6;7)(q13; q21.11),der(8)t(8;10)(q24.1;q21),der(9)t(3;9)(q12;p21)t(9;15)(q21;p15),t(9;16)(p21;q24),der(11)t(10;11)(?;q22),+der(11t(10;11) (?;q22)t(6;11)(q21;p15),+t(12;20)(q21;q13.3),der(13)t(13;?)(p11;?),−14,der(15)t(11;15)(?;p11)t(9;11)(q21;?),+16,+16, +der(16)t(3;16)(q27;q22),der(17)t(2;17)(q21.2;p11.1),+20,+20,der(21)t(8;21)(?;p11), +der(21)t(5;21)(p11;p11)ins(21;1) (q11.2;p21),+del(22)(q12.3),+dmin12 [cp 14]/106–108,<4n>,idemx2 [cp 2]

Table 1 (*continued*)

SW948	53–59,<3n>,X,X,−X,−1,+i(3)(q10),−4,der(5)t(5;17)(q11.1;q11.2),inv(5)(p15.3;q11),−6,i(8)(q10),inv(8)(p23;q13),−9,der(9;15)(q10;q10)del(15)(q11.2q24.1),−10,+13,−14,−15,−16,−17,−18,der(19)t(15;19)(q24.1;q13.31),+20,−21,−22 [cp 11]
SW1116	45–57,<2n>,X,+X,Y,der(1)t(1;1)(p36.1;q43),+2,+der(3;10)(q10;q10),+4,+5,+del(5)(p?)del(5)(q?),+der(7,15)(q10;q10),+del(7)(q11),i(8)(q10),+der(9;15)(q10;q10)del(15)(q11.2q23)t(9;9)(q34;q21.31)hsr(9)(q22),ider(9)(p10)dup(9)(p21.2),+t(9;13)(q21.31;q12)hsr(9)(q22),+der(11;19)(q10;q10),+der(13)t(13;13)del(13)(q31)del(13)(q13),der(14)t(14;15)(p11;q23),+der(16)t(7;16)(?;q11.2),+del(17)(p11.2),−18,del(19)(p12),+der(20)t(7;20)(?;p13) [cp 10]
SW1398	51–54,<2n>,X,−Y,+der(X)t(X;8)(p11.2;q24.1),+t(2;20)(p23;q11),+der(3;13)(q10;q10),+5,+der(7)t(1;7)(p36.1;q31),+der(8)t(4;8)(?;q22),+der(12)t(7;12)(q32;q15),+der(13)t(X;13)(p11.2;p11),i(14)(q10),+der(16)t(8;16)(q23;p13.1),der(18)t(9;18)(q22.2;q12.2)t(9;18)(?;p11.23)ins(9;18)(?;q22.1q23),+20 [cp 14]
SNU C1	36–89,<2n>,X,−X,del(8)(p12),+(8pter-8p10::9p10–p21.1::8p11.2–8p12::9p24.3-qter),+del(9)(p22.3p23),der(14)t(14;14)(p11;q24),+i(20)(q10),+del(20)(q11.2) [cp 7] /61–89,<4n>,idemx2 [cp 4]
SNU C4	45–46,X,Y,del(1)(p11–p22),t(8;12)(p11.2;q23) [cp 9]

FISH analysis of centromeres in translocations

The differences in centromeric involvement between adenocarcinomas and squamous cell carcinomas described above prompted us to investigate whether or not whole chromosome arm translocations found in adenocarcinomas originated by a different mechanism than those in squamous cell carcinomas. We performed FISH with centromeric probes for the chromosomes participating in whole chromosome arm translocations.

Seven of nine clonal whole chromosome arm translocations detected in adenocarcinoma cell lines showed only one of the two possible signals (Figure 2c), indicating that the centromere was derived from only one of the two participating chromosomes. The other two whole arm translocations showed two signals, indicating that (parts of) centromeres from both participating chromosomes were present. Conversely, in squamous cell carcinoma cell lines, all of 13 clonal whole chromosome arm translocations analysed showed two signals (Figure 2c).

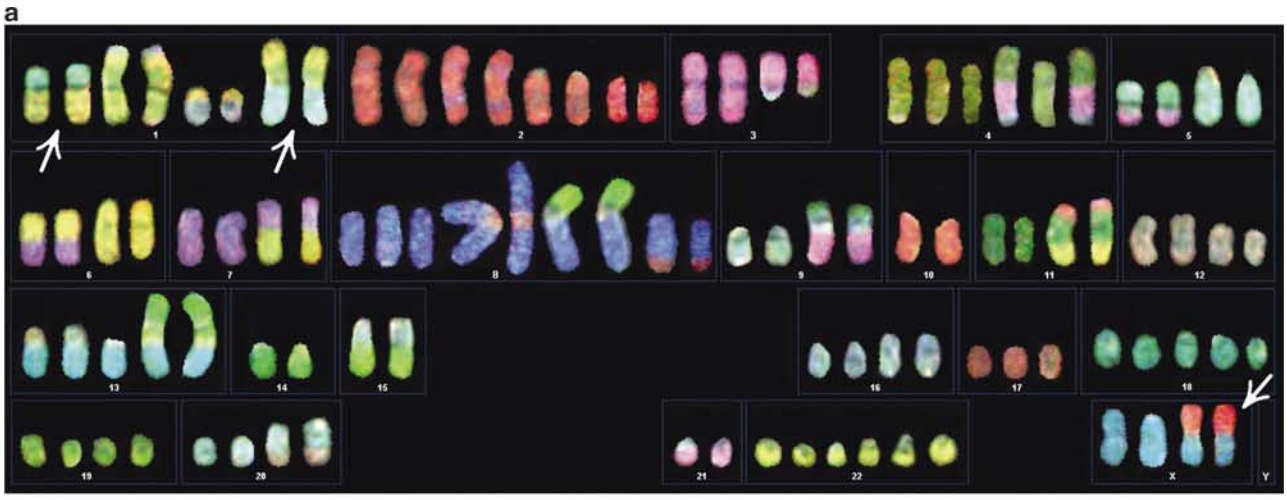
Structural aberrations in short-term squamous cell carcinoma culture

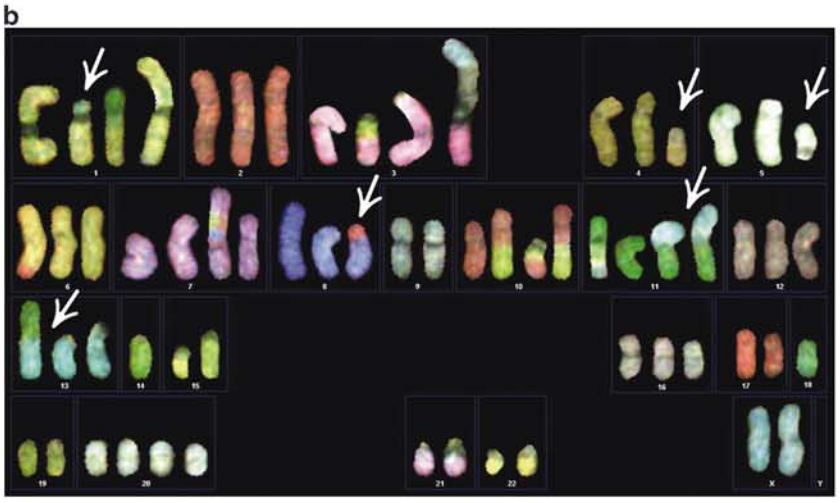
In order to rule out the possibility that the observed difference in the structure of translocations in squamous cell carcinomas compared to adenocarcinomas had arisen during prolonged time in culture, we analysed a short-term cell culture of a primary squamous carcinoma, scc1365. This tumor revealed a similar pattern of chromosomal abnormalities by SKY analysis as the previously studied squamous carcinoma cell lines. In all, 62% (10/16) of all breaks were centromeric, mainly consisting of whole chromosome arm translocations. Array CGH performed on DNA extracted from frozen tumor tissue showed the breakpoints of unbalanced whole chromosome arm translocations to be between the two BACs flanking the centromeres as demonstrated for chromosome 8 in Figure 2d. As in the squamous carcinoma cell lines, FISH experiments visualizing the centromeres of whole arm translocations of this short-term squamous carcinoma cell culture detected centromeric material of both participating chromosomes (Figure 2d). Thus, it is unlikely that the organization of centromeric rearrangements in the squamous cell carcinoma cell lines are artifacts of *in vitro* cell culture.

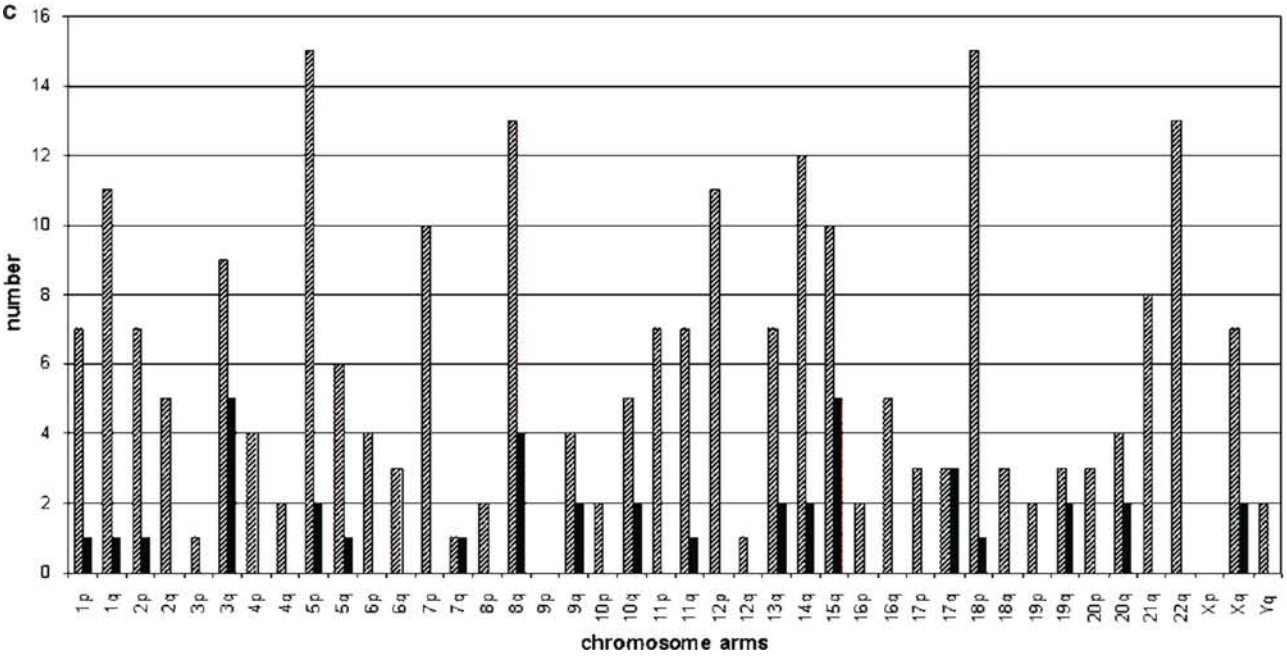
Discussion

In this study, we compared structural chromosomal aberrations in two epithelial tumor types, especially with regard to centromeric breakage. Results of combined information of complementary molecular cytogenetic techniques showed that squamous cell carcinomas, in contrast to adenocarcinomas, are unique with respect to breakage and fusion at (peri)centromeric regions, both in frequency and in location of the breaks. Knowledge of centromeres is still rather limited because of the high content of DNA repeat sequences. The majority of this repetitive DNA is present in centromeres of all chromosomes, making it very difficult to develop techniques that can study specific centromeres. For the squamous cell carcinoma cell lines, SKY, array CGH and FISH data indicate that chromosomal breaks and refusion frequently occur within the (peri)centromeric regions. Based on classical cytogenetic analysis and FISH, it has been suggested previously that centromere breakage and refusion is the mechanism of whole chromosome arm translocation in squamous cell carcinoma (Hermsen *et al*., 1996; Martins *et al*., 1999). In addition, Jin Y *et al*. (2000) also suggested

Figure 1 (**a**) Representative SKY karyotype of cell line Colo320, which is tetraploid and shows both numerical and structural chromosomal rearrangements. This cell line contains a balanced whole chromosome arm translocation t(1q;5p), and an unbalanced whole chromosome arm translocation (Xq;10q), indicated by arrows. (**b**) Representative SKY karyotype of cell line scc078, which is triploid and shows both numerical and structural chromosomal rearrangements. Amplification of chromosome 9 material can be seen at chromosome 3, between 3q and Xq. Arrows indicate the whole chromosome arm translocations, reading from 1 to X: (1q;18p), (4p;12p), (5p;18p), (8q;10p), 2x (11q;Xq) and (11q;13q). (**c**) Frequencies of participation of chromosome arms in whole chromosome arm translocations or isochromosomes, as detected by SKY. Black bars represent the seven adenocarcinoma cell lines and hatched bars the nine squamous cell carcinomas


a


b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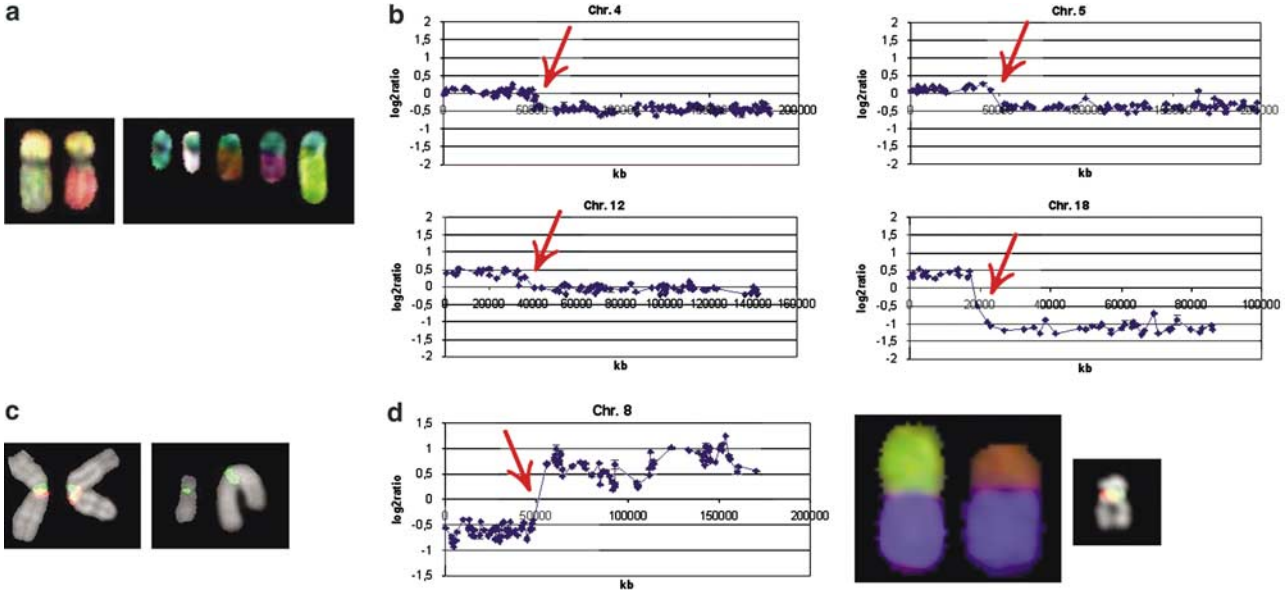

Figure 2 (**a**) Examples of chromosome arms frequently involved in whole arm translocations with different partners. Left: 22q is translocated to 1q and to 10q, in cell line scc1131. Right: 18p is translocated to 18p itself, to 20p, 4p, 12p and to 15q, in cell line scc120. (**b**) Whole arm translocations detected by SKY are in agreement with a change in copy number between the two BAC clones closest to the centromere, measured by array CGH. The left panel shows results of chromosomes 4 and 12, the right panel shows results of chromosomes 5 and 18, both from cell line scc078. Red arrows indicate the position of the centromere, which in these cases coincide with the transition in copy number. (**c**) FISH with centromeric probes shows whole chromosome arm translocations in squamous cell carcinoma containing two signals (left panel), and in adenocarcinoma containing one signal. Left panel left marker: der(1;10)(q10;q10) from scc120 shows green for centromere 10 and red for centromere 1, and left panel right marker: der(1;14)(p10;q10) from scc094 shows green for centromere 1 and red for centromere 14. Right panel left marker: der(17;19)(q10;q10) from cell line WiDr shows only one FISH signal of chromosome 17. Right panel right: der(3;13)(q10;q10) from sw1398 shows only one FISH signal of chromosome 13. (**d**) Results of the short-term culture scc1365 showing on the left whole arm imbalance of chromosome 8 as detected by array CGH (red arrow indicates the position of the centromere) and enlarged SKY images of the whole arm translocations in which chromosome 8 was involved: der(8;15)(q10;q10) and der(4;8)(p10;q10). On the right, FISH shows that whole arm translocation der(4;8)(p10;q10) contains centromeric material from both chromosomes

Table 2 Distribution of types of rearrangements, as detected by SKY

	Adeno-carcinoma cell lines (n = 7[a])	*Squamous cell carcinoma cell lines* (n = 9)	*Squamous cell carcinoma short-term primary culture* (n = 1)
Type of rearrangement	*n* = 114 translocations	*n* = 226 translocations	*n* = 16 translocations
Band–band	54%	31%	38%
Cen–band	21%	11%	0%
Cen–cen	25%	58%	62%
Cen–cen: isochromosome	14%	17%	6%
Cen–cen: whole arm	11%	41%	56%

[a]Cell lines WiDr and Colo205 were found to be derivatives of cell line HT29; therefore, they were regarded as one, making a total of seven cell lines; band–band: rearrangements in which the breakpoints of both partner chromosomes are located on the chromosome arms; cen–band: rearrangements in which one of the partner chromosomes is broken and fused within the arm and the other partner within the centromere; cen–cen: rearrangements in which both partner chromosomes are broken and fused within the centromere, leading to isochromosomes and whole arm translocations. The percentages of the two types of cen–cen rearrangements are given in the lowest row

that isochromosomes in skin and head and neck squamous cell carcinoma arise by cleavage of the centromere. The present data lend further support to this hypothesis. These studies, together with the data from the primary tumor after short-term culture, show that centromeric breakage and refusion are not cell line artifacts.

Although the resolution of the array CGH data used is very high, our results still do not exclude the possibility that the break is located in a small region on either chromosome arm between the centromere and the first juxtacentromeric clone on the array, which would approximately be at 3–5 Mbp distance. Such an event would also go undetected by FISH with centromeric probes for lack of resolution between the two signals (Figure 2c). Therefore, it cannot be fully excluded that (peri)centromeric breakage occurs close to, but not within the centromere. Still, both p- and q-arms of a specific chromosome were frequently involved in different whole arm translocations within individual cell lines, and both centromeres of the chromosomes involved were always present, which is consistent with the hypothesis that breakage actually occurs within the centromere.

In adenocarcinomas, the situation was different. Here, we mainly found a number of specific chromosome arms, that is, 3q, 8q, 15q and 17q, involved in whole arm translocations (Figure 1c). The fact that centromeric material of only one participating chromosome could be detected in marker chromosomes with

whole arm translocations at SKY resolution is consistent with the hypothesis that here breakage occurs close to, but not within the centromeres. This suggests that the mechanism involved here is probably the same as for other band–band translocations. Breakpoints in a gene close to the centromere could drive the selection of tumor cells with these 'whole arm' events. However, in squamous cell carcinomas, where in the case of the whole arm translocations the breakpoints are within the actual centromeres, it is unlikely that a specific gene drives the clonal selection of these translocations in a similar way as in adenocarcinomas, since centromeres are gene poor regions.

The mechanism for this putative intrinsic centromeric instability in squamous cell carcinomas is not known. Another form of centromeric instability has been described in ICF syndrome (immunodeficiency, centromere instability, facial abnormalities). In patients with this syndrome, isochromosomes and whole chromosome arm translocations are the main genetic feature and involve the paracentromeric heterochromatin of chromosomes 1, 9 and 16 (Sumner *et al*., 1998). Recently, the responsible gene was found to be *DNA methyltransferase 3B* (Xu *et al*., 1999). Mutations in both alleles render it inactive, leading to hypomethylation and decondensation of the paracentromeric heterochromatin of chromosomes, which makes the region more vulnerable to breakage. A similar process may cause breakage in the centromeric repeat sequences in squamous carcinomas.

Another mechanism involved may be loss of function of *topoisomerase II* that catalyses strand passage of double-stranded DNA. It is essential for chromosome condensation and sister chromatid exchange (Warburton and Earnshaw, 1997), and during metaphase it is associated with the centromere (Rattner *et al*., 1996). Two isoforms exist: *topoisomerase IIa*, mapped to 17q21–22 and expressed in developing tissues, and *topoisomerase IIb*, mapped to 3p24 and expressed in somatic tissues. It is interesting to note that squamous cell carcinomas show recurrent losses on chromosome 3p in up to 80% of the cases. Topoisomerases are the cellular target for DNA intercalating agents as well as nonintercalators, both interfering with the cleavage/religation step of *topoisomerase II*, resulting in a stabilization of the 'cleavable complex'. Accumulation of these complexes leads to inhibition of DNA replication, enhancement of sister chromatid exchange and chromosomal translocations (Bakshi *et al*., 2001). Experiments inhibiting the activity of *topoisomerase II* by the chemotherapeutic agent etoposide showed cleavage within the centromere, and that this was found only in active centromeres (Floridia *et al*., 2000).

The tissue specificity of the observed centromeric instability could be due to differences in the etiology of adenocarcinoma and squamous cell carcinoma. Squamous cell carcinoma of the head and neck, lung and esophagus all are related to tobacco smoking, while for adenocarcinomas, for example, of the large intestine, esophagus (Barrett) and lung, this relation is less obvious or absent. Compounds in tobacco could cause mutations or interfere with function of genes that maintain centromere integrity. In light of this, it is interesting to include investigations on tumors found in Fanconi anemia (FA) patients. FA patients are predisposed to specific malignancies, the second most frequent of which is squamous cell carcinoma (Joenje and Patel, 2001). It was recently shown that also squamous cell carcinomas from Fanconi patients show very frequent isochromosomes and whole chromosome arm translocations (Hermsen *et al*., 2001). Cells from FA patients show spontaneous chromosomal instability and are 10- to 100-fold more sensitive to intercalating agents, which may explain the early age of onset (20–30 years in Fanconi *vs* 60+ years in sporadic squamous cell carcinoma). Until now, however, it is unknown if FA gene defects play a role in sporadic squamous cell carcinoma development.

In summary, given its extraordinary high occurrence in this study and in previously reported cytogenetic studies on squamous cell carcinoma, irrespective of organ of origin, we propose that centromeric breakage and illegitimate recombination should be considered a critical type of chromosomal instability in the development of this type of epithelial cancer, in contrast to adenocarcinoma. We demonstrate for the first time, to our knowledge, tissue-specific differences of centromeric breakage followed by illegitimate recombination leading to whole arm translocations in epithelial cancer.

Materials and methods

Cell lines

All colon adenocarcinoma cell lines were kindly provided by Professor GJ Peters, Department of Oncology, VU University Medical Center, and were described previously (Van Triest *et al*., 1999). The oral squamous cell carcinoma cell lines were established by the first author in the Department of Human Genetics, VU University Medical Center, and have been karyotyped previously by cytogenetic analysis of Q-banded metaphase chromosomes (Hermsen *et al*., 1996). VU1365 was a short-term primary tumor cell culture of a poorly differentiated, stage 4 squamous cell carcinoma of the tongue, of which metaphase spreads were prepared of cells growing out from small explants after 7 days, before passaging.

Spectral karyotyping

SKY was performed according to the protocols described earlier (Schrock *et al*., 1999). In brief, tumor metaphase slides were pretreated with pepsin in order to remove the cytoplasm (Schrock *et al*., 2001). Slides were mounted with 100 μl denaturation mixture (70% formamide/2 $\times$ SSC, pH 7.0) and denatured on a hot plate at 73°C for 1.5 min, dehydrated in an ethanol series and air-dried. SKY probe preparation and labeling were performed via DOP–PCR (Telenius *et al*., 1992) of flow-sorted chromosomes (kindly provided by M Ferguson-Smith, Cambridge, UK) using Rhodamine 110-dUTP (Perkin-Elmer, Packard BioScience, Groningen, The Netherlands), Tamra-dUTP (Perkin-Elmer, Packard BioScience, Groningen, The Netherlands), Texas Red-dUTP (Molecular Probes, Leiden, The Netherlands), Biotin-16-dUTP (Roche Diagnostics, Almere, The Netherlands) detected with avidin-Cy5 (Amersham Pharmacia Biotech, Roosendaal,

The Netherlands) and Digoxigenin-11-dUTP (Roche Diagnostics, Almere, The Netherlands) detected with anti-digoxin (Sigma) and a Cy5.5 anti-mouse antibody (Amersham Pharmacia Biotech, Roosendaal, The Netherlands). The SKY probe mixture was precipitated together with 50 μg Cot-1 DNA and 10 μg salmon sperm DNA, taken up in 12 μl hybridization mixture (50% formamide, 10% dextran sulfate in 2 × SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0), denatured for 5 min at 80°C, preannealed at 37°C for 1 h and mounted on the metaphase slides. Hybridization took place for 2 days in a humid chamber at 37°C. Detection was performed as described previously (Schröck *et al*., 1999). Images were acquired using a Leica DMRA fluorescence microscope equipped with the SpectraCube™, and analysed with the SpectraView software, version 1.6.1 (Applied Spectral Imaging Inc., Migdal Haemek, Israel).

Array CGH

Experiments were carried out as described in detail by Snijders *et al*. (2001) using arrays of 2464 BAC clones printed in triplicate to create an array of ~7500 elements in a 12 mm^2 area (HumArray1.4). Tumor and reference DNA were labeled by random priming and 300 ng each of test and reference DNA were hybridized to the arrays. Image acquisition and analysis was carried out using a custom-built CCD camera imaging system (Pinkel *et al*., 1998). Analysis of the array images with automatic feature extraction and subsequent data analysis was performed with dedicated software SPOT and SPROC (Jain *et al*., 2002). Fluorescence ratios of clones for which only one of the triplicate values remained after SPROC analysis or for which the standard deviation of the log 2 ratios of the triplicate was >0.2 were excluded from the analysis (Snijders *et al*., 2003).

Fluorescence in situ *hybridization*

FISH analysis was carried out using centromere-specific DNA probes, directly labeled by PCR with Rhodamine 110-dUTP (Perkin-Elmer, Packard BioScience, Groningen, The Netherlands), Spectrum Orange-dUTP (Vysis Downers Grove, IL, USA) or Texas Red-dUTP (Molecular Probes, Leiden, The Netherlands), hybridizing to the α-satellite regions of human centromeres. For chromosomes X, 3, 7, 10 and 18, commercial centromeric probes were used that were labeled with Spectrum Green and Spectrum Orange (Vysis, Downers Grove, IL, USA). DNA probe mixtures in hybridization buffer and chromosome slides were denatured as described in the CGH section. After hybridization for 24 h at 40°C, the slides were washed for 5 min in 2 × SSC at RT, three times 5 min in 0.1 × SSC at 45°C, and then dried in an ethanol series. Finally, the slides were mounted with 20 μl antifade solution (Vectashield, Vector laboratories, Burlingame, USA) containing 0.35 μg/μl 4,6-diamidino-2-phenylindole (DAPI). The slides were analysed on a Leica DM-RA microscope equipped with filter sets for DAPI, FITC and TRITC.

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