# Similar regions of human chromosome 3 are eliminated from or retained in human/human and human/mouse microcell hybrids during tumor growth in severe combined immunodeficient (SCID) mice

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**By passaging microcell hybrids (MCHs) containing human chromosome 3 (chr3) on A9 mouse fibrosarcoma background through severe combined immunodeficient (SCID) mice (elimination test), we have previously defined a 1-Mb-long common eliminated region 1 (CER1) at 3p21.3, a second eliminated region (ER2) at 3p21.1-p14 and a common retained region (CRR) at 3q26-qter. In the present work, chr3 was transferred by microcell fusion into the human nonpapillary renal cell carcinoma line KH39 that contained uniparentally disomic chr3. Four MCHs were generated. Compared with KH39, they developed fewer and smaller tumors, which grew after longer latency periods in SCID mice. The tumors were analyzed in comparison with corresponding MCHs by chr3 arm-specific painting, 19 fluorescent *in situ* hybridization (FISH) probes, and 27 polymorphic markers. Three MCHs that maintained the intact exogenous chr3 *in vitro* lost one 3p copy in all 11 tumors. Seven of 11 tumors lost the exogenous 3p, whereas four tumors contained mixed cell populations that lacked either the exogenous or one endogenous KH39 derived 3p. In one MCH the exogenous chr3 showed deletions within CER1 and ER2 already *in vitro*. It remained essentially unchanged in 8/9 derived tumors. The third, exogenous copy of the 3q26–q27 region (part of CRR) was retained in 16/20 tumors. It can be concluded that the human/human MCH-based elimination test identifies similar eliminated and retained regions on chr3 as the human/murine MCH-based test.**

In collaboration with Henry Harris, we have shown that fusion of normal cells with malignant cells has led to regular suppression of tumorigenicity as long as the hybrid chromosome complement was maintained (1). Suppression of tumorigenicity was not associated with impairment of *in vitro* growth. Following the loss of normal parent-derived chromosomes, tumorigenicity reappeared. Stanbridge and colleagues (2) showed subsequently that tumorigenicity can also be suppressed by the introduction of single normal cell-derived chromosomes via microcell fusion. Reappearance of the tumorigenic phenotype was associated with the loss of the transferred chromosome or its parts.

To analyze the role of one of the most frequently deleted regions in human tumors, which is located on the short arm of human chromosome 3 (chr3), we have generated microcell hybrids (MCHs) with the malignant mouse fibrosarcoma cell A9 as the recipient. Measuring the suppression of tumorigenicity caused by the introduced chromosome is difficult however, because the exogenous chromosome or parts of it keep being eliminated. We have therefore chosen to identify regularly eliminated regions instead. In contrast to the intact chr3 carried by most MCHs *in vitro*, severe combined immunodeficient (SCID)-derived tumors contained only human chromosome fragments translocated to various mouse chromosomes. Marker analysis showed that a ≈43-cM segment was missing from all tumors. Studying additional tumor panels, the common eliminated region was narrowed down to about 1 Mb [designated common eliminated region 1 (CER1)] and covered by a single PAC contig (3–6). A second eliminated region, missing from most but not all tumors, has been found at 3p21.1-p14 and designated second eliminated region (ER2) (4). In addition to the eliminated regions, we could also identify a common retained region (CRR) of ≈40 cM, at 3q26-qter. It was regularly retained in 85/92 tumors and was present even after 4 consecutive SCID–SCID passages (7).

So far all elimination tests were performed on mouse A9 fibrosarcoma-based MCHs carrying a single human fibroblast-derived chr3. In the present study we have set out to check the validity of the elimination test on human chr3/human tumor MCHs. We have chosen the nonpapillary or clear cell type of renal cell carcinoma (RCC), where 3p losses occur in most tumors (8, 9). We have transferred a normal human chr3 from a monochromosomal chr3/mouse A9 fibrosarcoma hybrid (A9-Hytk3) into the human nonpapillary RCC line KH39. We have established four *in vitro* propagated MCHs (designated YYKs) and analyzed the chr3 changes after tumor growth in SCID mice.

## Materials and Methods

**Cell Lines and Microcell-Mediated Chromosome Transfer.** A9Hytk3 is a microcell hybrid that contains a single cytogenetically intact human chr3 on mouse fibrosarcoma A9 background (10). It was used as a chr3 donor by microcell fusion as described (2). The conventional (nonpapillary or clear cell) renal cell carcinoma line KH39 served as the recipient. Microcell hybrid lines were selected on 400 μg/ml Hygromycin B (Sigma). Four MCHs, designated as YYK1, -2, -3, and -4, were obtained from four different fusion events.

**Tumorigenicity Tests.** Six-week-old SCID mice were used for the inoculations ($10^6$ cells per site). Both single-site and four-site

Abbreviations: CER1, common eliminated region 1; chr3, human chromosome 3; CRR, common retained region; ER2, second eliminated region; MCH, microcell hybrid; FISH, fluorescent *in situ* hybridization; cM, centimorgan; SCID, severe combined immunodeficient; RCC, renal cell carcinoma; CGH, comparative genomic hybridization.

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inoculations were performed. In the latter case, each mouse received three MCH samples and one parental RCC (KH39) control. SCIDs were observed for tumor formation once a week up to 20 weeks. The tumors were excised under sterile conditions, explanted, and expanded for chromosome and DNA preparations.

**Polymorphic Marker Analysis.** Microsatellite markers were either end-labeled by [$\gamma$-$^{32}$P]dATP using T4 polynucleotide kinase (Amersham Pharmacia) or by fluorescent (6-FAM, HEX, and TET) dyes. One [$\gamma$-$^{32}$P]dATP end-labeled primer (0.2 pmol) was used for PCR in a 20 $\mu$l volume containing 50–100 ng template DNA, 2 pmol of each primer, 0.2 mM dNTP, and 1.7 units *Taq* DNA polymerase (Amersham Pharmacia). The PCR program was as follows: 95°C for 5 min, 30 cycles of 3 steps (95°C for 30 sec, 55°C for 1 min, and 72°C for 1 min), and 72°C for 7 min for the last extension. PCR reaction (1–2 $\mu$l) was separated with 6% denatured polyacrylamide gel and the gel was exposed to the x-ray film. Signals were visualized after 24 h. Fluorescent dye-labeled primer (2 pmol) was used in a 10-$\mu$l volume containing 5–10 ng template DNA, 0.2 mM dNTP, and 0.5 unit of AmpliTaq Gold (Perkin–Elmer). The PCR program was: 95°C for 10 min, 30 cycles with 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec; final elongation 72°C for 7 min. PCR products (1–2 $\mu$l) from each reaction were grouped according to the sizes of products and separated with 5% denatured polyacrylamide gel on ABI 377 (Perkin–Elmer) using GENE SCAN software. Allele sizes were determined by GENOTYPER 2.1 (Applied Biosystems). Some primers were elongated by using terminal-transferase and covalently labeled with peroxidase and used to probe membranes after transfer from the gel (ECL kit; Amersham Pharmacia) (11).

**Allele Band Densitometry.** A Molecular Dynamics Personal Densitometer SI (Amersham Pharmacia) was used to measure the density of allele bands.

**Long Genomic Fragment Amplification.** To prepare fluorescent *in situ* hybridization (FISH) probes for the analysis of the lactotransferrin (LTF) gene, the Expand Long Template PCR System kit (Roche Molecular Biochemicals) was used. The LTF exon 6–12 primers were: forward, 5′-CTGGAGACGTGGCTTTTATCAG-3′; reverse, 5′-CTTGCCTTTCACAGATTCAG-3′. PCR conditions were designed as suggested by the manufacturer. DNA from PAC RP6–19p19 was used as a template. The size of product was ≈9 kb (designated LTF 6–12).

**FISH.** Probes were labeled by nick-translation with either biotin-dUTP (Bionick labeling system, BRL) or digoxigenin-dUTP (DIG-Nick Translation Mix, Roche Molecular Biochemicals). Chr3 arm-specific painting was performed by using MCH B78-MC56, which contains a t(3p;17q) on murine B78 melanoma background (kind gift of Mario Chevrette, McGill University, Montreal). B78-MC56-derived DNA was labeled with biotin and detected by Cy3 conjugated avidin (red); MCH A9Hytk3 (carries intact chr3)-derived DNA was labeled with digoxigenin and detected by FITC-conjugated anti-digoxigenin antibodies (green). Simultaneous FISH with these two probes painted 3p in yellow, 3q in green, and 17q in red. Commercial chr3 painting and centromere-specific probes labeled with fluorescein or Cy3 (Cambio, Cambridge, U.K.) were also used. One- and two-color FISH was performed on metaphases and interphase nuclei prepared from methanol/acetic acid fixed cell suspensions as described (12). At least 20 metaphases and 50 nuclei were analyzed for each sample.

Comparative genomic hybridization (CGH) was performed as described (13).

**Databases and Software.** PCR markers were selected mainly from the following databases: the genome database (GDB, www.gdb.org); an STS-Based Map of the Human Genome (14) from the Whitehead Institute (www.genome.wi.mit.edu/cgi-bin/contig/phys-map). BLAST programs were used for database searches on the National Center for Biotechnology Information (NCBI)/National Institutes of Health (NIH) server (www.ncbi.nlm.nih.gov/cgi-bin/BLAST). The PRIMERS software (www.williamstone.com/primers/) was used for primer selection.

## Results

**Characterization of the Recipient KH39, Donor A9Hytk3, and the chr3/KH39 MCHs: YYK1, -2, -3, and -4.** In KH39, analysis of 35 polymorphic markers on 3p, 20 of which were published earlier (13), and nine 3q markers has shown that the whole chr3 was hemizygotic. In A9Hytk3 we have checked the intactness of the human chr3 by 54 PCR markers (ref. 10 and unpublished data). Cytogenetic methods, including G-banding, FISH painting, chr3 centromere probe, and 3p locus-specific probes on metaphases and interphase nuclei, were used to monitor the presence and intactness of transferred chr3. Two intact copies of chr3 were found in KH39, one in A9Hytk3, and three in YYK2, -3 and -4 (Fig. 1*a*). In YYK1, the third copy of chr3 was rearranged. The position of the centromere, banding pattern, and arm-specific painting revealed a pericentric inversion accompanied by a deletion(s) (Fig. 1*c*). CGH analysis of YYK1 vs. KH39 showed a uniform 3p gain, although FISH with 19 locus-specific probes detected two 3p deletions (Fig. 2). One of them overlapped partially with CER1, whereas the second mainly corresponded to ER2. G-banding, 3q painting, and CGH analysis suggested also large 3q11–q21 and 3q27-qter deletions.

**Tumorigenicity Tests.** SCID-grown KH39 tumors had an average take incidence of 86% after inoculation of $10^6$ cells. As shown in Table 1, YYKs had a mean take incidence of 39% (61 tumors per 155 inoculations). The latency time of the YYK tumors was 3–5 weeks, in contrast to KH39, which was regularly palpable after 1 week. The mean size of all tumors was significantly smaller (only 4/20 exceeded 1 cm$^3$) compared with KH39 tumors, which reached 0.5–2.4 cm$^3$ at the time of explantation. Only 20 of 61 YYK tumors were explanted successfully, in contrast to the regularly explantable KH39.

**Polymorphic Marker Analyses of Tumors.** Fifty chr3-specific polymorphic microsatellite and restriction fragment length polymorphism (RFLP) markers were tested for their ability to differentiate between the exogenous A9Hytk3-derived chr3 and the endogenous chrs3 of the KH39. Altogether, 18 3p markers (including three CER1 and two ER2 markers) and nine 3q markers were informative. Twenty YYK-derived tumors were analyzed (Figs. 3 and 4*a*). The tumors could be divided in three groups (g, I–III)—g, I: tumors identical with the *in vitro* MCH [YYK1 tu2–tu9; these had two deletions on 3p that affected the exogenous chr3-derived alleles of D3S2354 (CER1), D3S1573, and D3S1076 (ER2)]; g, II: tumors that lost most (YYK1 tu1 and YYK2 tu1 and tu2) or all exogenous 3p alleles (YYK2 tu3, YYK3 tu3, and YYK4 tu3–tu5); g, III: tumors that retained all exogenous alleles (YYK2 tu4 and tu5 and YYK3 tu1 and tu2).

Three CRR (7) markers, D3S1763, D3S3053, and 3S1262, remained heterozygous in all YYKs and in 16/20 tumors. Three YYK4 derived tumors that lost the whole exogenous chr3 and one YYK1 tu1 that lost all but four chr3 markers were the exceptions.

**FISH Analyses of Tumors.** We have chosen representative tumors of each group—g, I: YYK1 tu2, tu3, and tu4; g, II: YYK2 tu1, tu2, and tu3 and YYK4 tu3 and tu4; and all tumors from g, III. The

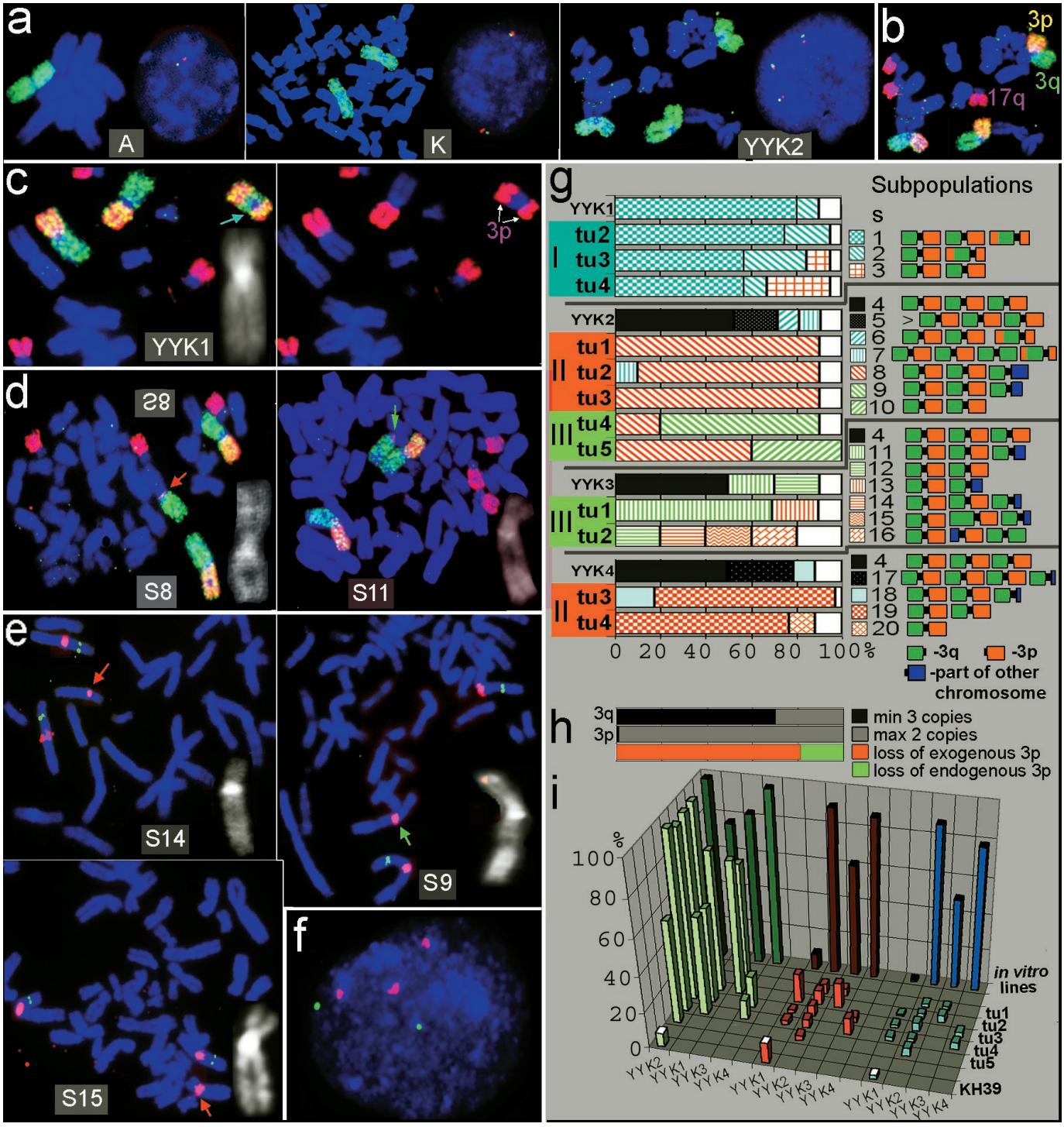


**Fig. 1.** FISH analysis of chr3 in A9Hytk3, KH39, YYKs, and 12 YYK-derived SCID tumors (groups I–III). Subpopulations (S1–20) were identified according to the number and morphology of chrs3 (rearranged chromosomes are marked by arrows and magnified as black and white DAPI-banded images at the corner of boxes). (*a*) Chr3 painting (green) and interphase FISH (red, RP6–19p19; green, Rp6–102f2) analyses of chr3 copy number in cell lines: A, A9Hytk3 (donor of chr3); K, KH39 (recipient); and YYK2 (one of the generated MCHs). (*b*) Arm-specific painting as described in *Materials and Methods* for the same metaphase of YYK2. (*c*) Pericentric inversion in YYK1. (*d*) Loss of 3p in tumor cells (S8, S11). (*e*) Loss of CER1 on rearranged chr3 in tumors (S14, S15, S9) shown by FISH with chr3 centromere (red) and CER1 PAC 188 g11 (green) probes on metaphases. (*f*) Loss of the third copy of CER1 detected by interphase FISH in YYK2 tu1 (same probes as in *e*). (*g*) Percentage of different cell subpopulations (S1–S20) in YYKs and derived tumors. Black patterns on bars: minimum three intact chrs3. Color patterns: maximum two intact chrs3. Blue patterns: exogenous 3p rearranged *in vitro* (blue arrow in *c*). Red patterns: exogenous 3p lost *in vivo* (red arrows in *d* and *e*). Green patterns: endogenous 3p loss (green arrows in *d* and *e*). White: other minor (<10%) subpopulations. (*h*) Retentions vs. losses on 3q and 3p (black vs. gray, counted from g, II and g, III, results shown in *g*). Ratio of exogenous vs. endogenous 3p losses (red vs. green, counted from total exogenous 3p losses in g, II and red vs. green bars in g, III shown in *g*). (*i*) Percentage of cells with minimum three signals of: chr3 centromere (green) and CER1 PAC 188 g11(red) (based on interphase FISH as illustrated in *c*); intact chrs3 (blue) (based on metaphase FISH, shown in *a* and *b*) in YYK *in vitro* lines, derived tumors, and original KH39 line.

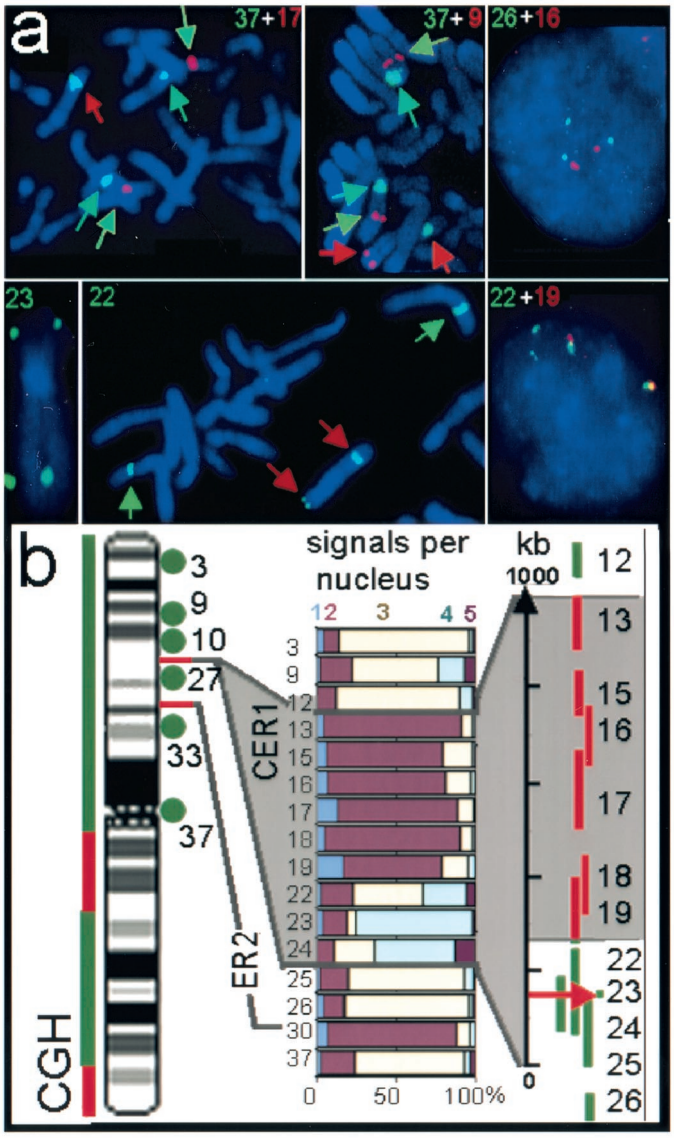


**Fig. 2.** FISH mapping of 3p deletions in YYK1. Probes are shown by numbers, the corresponding names and locations are listed in fig 3. (*a*) Metaphase and interphase FISH. Probe number and color as indicated. Signals on the rearranged chr3 are marked by red arrows, on the normal chromosomes by green arrows. (*b*) From left to right: CGH of YYK1 vs. KH39—gain over 3p and 3q21–27 (vertical green bars) and no gain (large deletions in additional chr3) in the regions marked by vertical red bars. Green circles indicate retained FISH probes on the rearranged chrs3, red lines show the positions of deleted CER1 and its borders (12 probes) and ER2 (deleted probe 30). Horizontal bars: % of nuclei with 1–5 signals for different FISH probes. Final map of the CER1 deletion in kb scale (see ref. 6). Red lines indicate deleted probes; green lines indicate retained probes; and red arrow indicates disrupted probes (probes 22, 23, and 24).

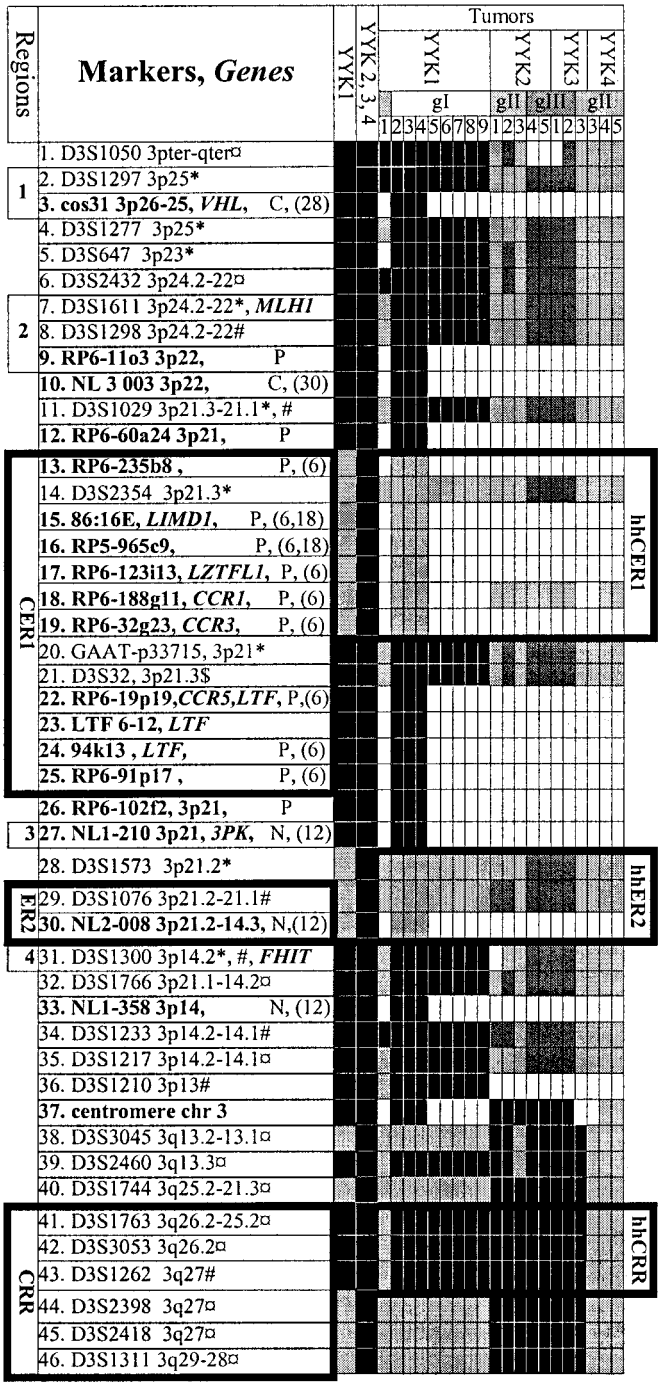


**Fig. 3.** Polymorphic (normal letters) and FISH (bold) marker analysis of YYKs and 20 derived tumors divided in 3 groups (gI–gIII). Italics, relevant genes. Microsatellite marker labeling: * by $^{32}$P, ¤ by fluorescence, # by peroxidase (ECL kit). $, restriction fragment length polymorphism (RFLP) marker; C, cosmid; P, PAC; N, *Not*I clone (references). Black boxes: two alleles or minimum three FISH signals per cell. Light gray boxes: LOH or maximum two FISH signals per cell. Dark gray boxes: two alleles, but loss of one 3p copy by FISH. White boxes: not done (in YYK2 tumors, YYK3 tu1 and tu2 and YYK4 tu4 and tu5 most of FISH markers were not tested, because painting has shown an entire 3p copy loss).

tumors analyzed by morphology and copy number of chr3 were found to contain 20 subpopulations (S1–S20 in Fig. 1 *d*, *e*, and *g*). Chr3 arm-specific painting (Fig. 1*b*) detected two (sometimes one) copies of intact chr3 (3p arm) in the majority of the cells (Fig. 1 *d*, *g*, and blue bars in *i*) in all three groups and their subpopulations. The third 3p was always lost (g, II and g, III) or rearranged (g, I). For CER1 analysis, we used a combination of centromere probe and PAC RP6–188 g11, located in the middle of CER1. We detected chr3 centromere, but no PAC signal on the rearranged chrs3 (Fig. 1*e*). By interphase FISH (Fig. 1*f*), all tumors had only two RP6–188 g11 signals (Fig. 1*i*, red bar), whereas the centromeres were maintained in three copies (Fig. 1*i*, green bars), except YYK4 tu3 and tu4, which have eliminated an entire chr3.

In agreement with polymorphic marker analysis in g, I, we

**Table 1. Tumorigenicity of KH39 and its chr3 MCHs YYK1-4 in SCID mice ($10^6$ cells inoculated)**

| Cells | Tumors/ site | Take incidence, % | Mean latency, weeks | Successful explants, % |
|---|---|---|---|---|
| KH39 | 25/29 | 86 | 1 | 100 |
| YYK1 | 26/84 | 31 | 5 | 35 |
| YYK2 | 15/34 | 44 | 5 | 33 |
| YYK3 | 16/22 | 73 | 3 | 19 |
| YYK4 | 4/15 | 27 | 4 | 75 |

found no further significant changes compared with the *in vitro* subpopulations (blue bars in Fig. 1*g*).

In a contrast to the LOH data, g, II and III did not differ by FISH analyses. In both groups, loss of additional 3p copies occurred in virtually 100% of cells, but 70% of cells still retained 3q (Fig. 1*h*). The heterozygosity was maintained in *g*, III, indicating that an endogenous 3p was eliminated.

**Analysis of Exo-/Endogenous 3p Losses.** The tumors of g, III were most heterogeneous, suggesting complex exo-/endogenous 3p losses. Fig. 4*b* shows the densitometric analysis of four polymorphic markers in these tumors. The average ratio of two alleles indicates no significant difference between the *in vitro* lines and YYK2 tu4 and tu5, suggesting that the 3p loss found on cytogenetic examination affected both endogenous and exogenous chromosomes. In these tumors the S9 and S10 were different from the S8, which was dominant in the YYK2-derived tumors of group II. Therefore we consider that the S8 has lost the exogenous 3p, whereas S9 and S10 have lost the endogenous 3p. In YYK3 tu1 the endogenous allele decreased from 77.1% to 55.2%, suggesting a significant endogenous 3p loss on SCID

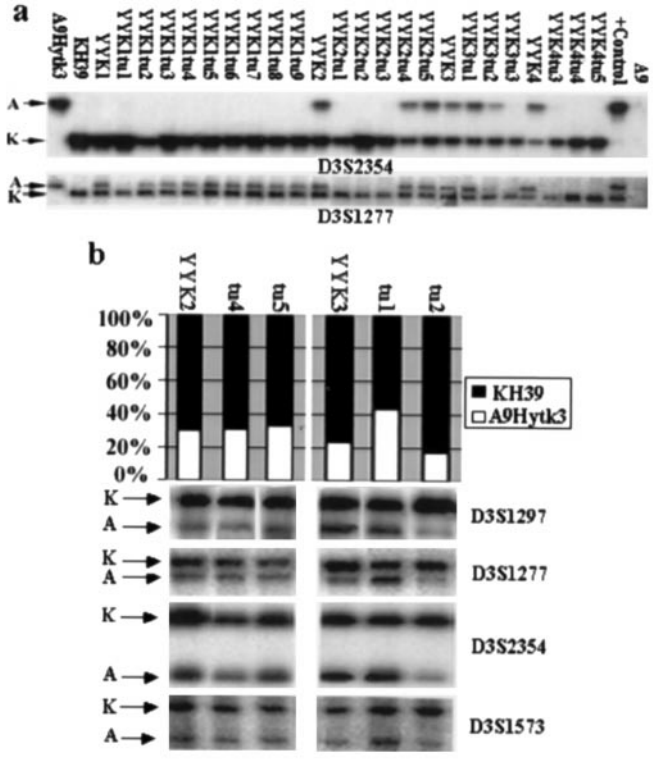


**Fig. 4.** (*a*) Microsatellite marker analysis with CER1 (3p21.3) marker D3S2354 and 3p25 marker D3S1277. A, A9Hytk3; K, KH39. (*b*) Average band density ratios for four microsatellite markers. K, KH39 allele; A, A9HytK allele.

passage, which could occur in S11. In YYK3 tu2 the exogenous allele density was reduced, suggesting that a minority of cells lost endogenous 3p. S12, which had only two chr3 copies, was also present in the YYK3 line. Because this was hygromycin-resistant, the lost copy should be an endogenous chr3. The "zebra pattern" of exogenous allele losses in YYK2 tu1 and tu2 (Fig. 3) may be related to the pericentric inversion and interstitial deletions on the rearranged chr3 (S7) in 10% or less of cells.

We may conclude that in g, III, loss of endogenous 3p has occurred in 20–70% of cells (green bars in Fig. 1*g*). The rest of the cells eliminated exogenous 3p (red bars in Fig. 1*g*). In all, the 11 tumors of g, II and g, III lost exogenous 3p in ≈80% of cells and an endogenous 3p in ≈20% of cells (Fig. 1*h*).

**Fine Mapping of CER1 Deletion in YYK1 and Derived Tumors.** Metaphase and interphase FISH (Fig. 2), as well as polymorphic marker analyses (Fig. 3), detected deletions inside CER1 and ER2 in YYK1 and derived tumors. Five PACs from CER1 and NL2–008 from ER2 were deleted on the rearranged chr3. RP6–32 g23 (probe 19 in Fig. 2) gave an unusually small hybridization signal. Both PAC 94k13 (probe 24) and RP6–19p19 (probe 22), which contained a full length LTF gene, were disrupted by the pericentric inversion. We have used two additional FISH probes, RP6–91p17 (probe 25), containing exons 1–6 of LTF, and a large size LTF fragment generated by PCR (see *Materials and Methods*), including exons 6–12 (probe 23), for further analysis of the gene. The latter detected a breakpoint within the exon 6–12 region. As summarized in Fig. 2*b*, the deletion is spanning over ≈700 kbp of CER1.

## Discussion

CER1 and ER2 on 3p and CRR on 3q have been identified by passaging the murine A9 fibrosarcoma cells that carried a single, normal fibroblast-derived human chr3 in SCID mice (4, 5, 7). Now, we tested whether the same regions were affected when a human chr3 hemizygous tumor cell (the RCC line KH39) was used as the recipient of a normal chr3. By tumorigenicity tests we found that the introduction of a normal chr3 reduced the take incidence of KH39 and prolonged the latency period, in line with earlier findings (reviewed in refs. 15 and 16). In contrast to earlier data (17), however, *in vitro* growth was not inhibited. These findings were consistent with our earlier findings with somatic cell hybrids derived from the fusion of normal and malignant cells that grew *in vitro* as well as the parental tumors, but were low or nontumorigenic *in vivo*.

The analysis of the successfully explanted tumors has shown that: (*i*) All 11 tumors derived from MCHs YYK2, -3, and -4 that carried an intact exogenous chr3 before inoculation eliminated one 3p copy after SCID passage; (*ii*) all nine tumors derived from YYK1, which carried an exogenous chr3 with CER1 and ER2 deletions *in vitro*, grew in SCID mice without further chromosome changes; and (*iii*) exogenous 3q26-q27 was retained in 16/20 tumors.

These findings indicate that 3p regions present in YYK2, -3, and -4, but absent in YYK1 (hhCER1 and hhER2) may complement 3p-associated genetic defect(s) that is mandatory for the tumorigenicity of KH39.

The telomeric border of CER1 was the same in the human/human and human/murine systems. The centromeric border of hhCER1 is ≈250 kb more distal. It contains the genes *KIAA0028*, leucyl-tRNA synthetase and *LIMD1*, LIM domain containing 1 (18), *CCR1, CCR3,* CC-chemokine receptors (6), *KIAA0851/SAC1*, suppressor of actin in yeast *LZTFL1*, leucine zipper transcription factor-like 1, *XT3*, orphan transporter and *CCR9*, and CC-chemokine receptor 9 (H.K. *et al.*, unpublished observations). *LTF* (lactotransferrin) or *LF* (lactoferrin) was disrupted in YYK1 by the inversion on the exogenous chr3. LTF was reported to inhibit the growth of methylcholantrene-induced mouse fibrosarcoma cells and v-ras-transformed NIH 3T3 cells *in vitro*. It has also reduced the

frequency of experimental metastases of melanoma cells in mice (19). An alternatively spliced form, delta lactoferrin (Δ*LF*), is transcribed in a variety of normal adult and fetal human tissues, but not in 14 tumor-derived cell lines (20). Marker D3S32, located on the same PAC RP6–19p19 as LTF, showed loss of heterozygosity in 9/14 and 17/21 analyzed nonpapillary RCCs (21, 22), and in several other solid tumors (5).

The telomeric border of the ≈15 centimorgan (cM) ER2 in the human/human MCHs (hhER2) is more proximal, because its border marker, D3S1573, is deleted in YYK1. The centromeric border is close to, but excludes *FHIT* (fragile histine triade gene) at 3p14.2. LOH was found in ER2 in RCCs (21, 23, 24), NSCLCs (25), and cervical carcinomas (26).

Several other regions (marked 1–4 in Fig. 3) are known to be involved in segmental 3p losses (27): (*i*) 3p26-p25, which contains the tumor suppressor gene *VHL1,* Von Hippel-Lindau gene (28); (*ii*) 3p24-p22, which contains the mismatch repair gene *MLH1*, and includes a *3p22* region involved in homozygous deletions in NSCLC (29); (*iii*) 3p21.1, a second region with multiple homozygous deletions detected in SCLC and breast carcinoma located between CER1 and ER2 immediately centromeric to the *3PK,* MAP kinase-activated protein kinase (30); and (*iv*) at 3p14.2, putative tumor suppressor gene *FHIT* is known to be inactivated in many human tumors and to be impaired in 34/34 SCID-passaged human/mouse MCHs in our system (31). None of these regions were deleted in YYK1-derived tumors.

Four SCID passages of human chr3/murine MCHs also identified a CRR at 3q26-qter, spanning over 43 cM between D3S1282 and D3S1265 (7). In the hhCRR (3q26–q27), D3S1763, D3S3053, and D3S1262 retained heterozygosity in 16/20 YYK-derived tumors. The four exceptions have either lost the whole exogenous chr3 or most of it. At the single cell level, FISH has shown that 70% of the cells that have lost 3p in tumors have retained 3q. In human tumors such as SCLC, cervical, ovarian, bladder, kidney, and breast carcinomas, 3q isochromosomes, duplications, and CGH-detected gains over 3q are frequently found (32, 33). Seven of nine RCC cell lines showed CGH gains over 3q26-q27 in our experience (ref. 13 and Kost-Alimova, unpublished observations). These findings suggest that genes located in 3q26-q27 can provide tumor cells with a selective growth advantage.

In conclusion, hhCER1 and hhER2 have been eliminated from all 20 tumors examined in this study. The transferred exogenous chr3 was lost in 16 tumors. In the remaining four tumors, the situation was more complex. The exogenous 3p was eliminated from a subpopulation of cells, whereas one of the two endogenous 3p was lost in the rest. In all, YYK2, -3, and -4-derived tumors, the ratio of exo- to endogenous 3p losses was ≈80% to ≈20%. If the losses occurred randomly, this ratio would be 1:2, because YYKs contain one exogenous and two endogenous copies of chr3. Loss of the exogenous 3p is thus clearly preferred, but gene dosage effects, perhaps in combination with epigenetic inactivation, may also modulate the loss of the tumor antagonizing function attributed to 3p. This is consistent with the fact that human solid tumors frequently show 3p losses, but rarely gains, as judged by CGH (33).

Our results on the human chr3/mouse tumor and the human chr3/human tumor cell MCHs converge, indicating that the elimination of hhCER1 and hhER2 is regularly associated with tumor growth. It is noteworthy, however, that YYK1 has lost these regions already *in vitro* and showed a reduced take incidence and prolonged latency period *in vivo*. This suggests that 3p genes outside our eliminated regions may antagonize tumor growth as well.

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