RESEARCH

The Human *CAS* (*Cellular Apoptosis Susceptibility*) Gene Mapping on Chromosome 20q13 Is Amplified in BT474 Breast Cancer Cells and Part of Aberrant Chromosomes in Breast and Colon Cancer Cell Lines

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The *CAS* (*cellular apoptosis susceptibility*) gene is the human homolog of the yeast chromosome segregation gene *CSE1*. *CAS* may have a dual function in mammalian cells, one in apoptosis and another in cell proliferation. We have now mapped the *CAS* gene to chromosome 20q13. This region is known to harbor amplifications that correlate with aggressive breast cancer. Southern hybridizations with a *CAS* cDNA fragment and fluorescent in situ hybridization (FISH) with a PI clone containing the *CAS* gene show elevated copy numbers in one leukemia, three of four colon, and in three of seven breast cancer cell lines. Elevated *CAS* copy number in CEM leukemia and COLO201 colon cancer cells was attributable to additional copies of chromosome 20. In SW480 and COLO205 colon cancer cells *CAS* is part of aberrant chromosomes containing large parts of 20q. In breast cancer cells *CAS* is also part of aberrant 20q chromosomes (MDA-MB-157 and UACC-812) or of additional 20q isochromosome in MDA-MB-134. In MDA-MB361 and BT-474 breast cancer cells *CAS* is separated from other markers centromeric and telomeric of *CAS* on 20q. MDA-MB 361 contains one additional copy of *CAS*, separated from the centromeric 20q control probe. BT-474 cells have up to 12 additional *CAS* copies that we separated from nearby telomeric and centromeric probes on 20q and that are translocated to abnormal chromosomes.

The *CAS* (*cellular apoptosis susceptibility*) gene is the human homolog of the essential yeast chromosome segregation gene *CSE1* (Xiao et al. 1993; Brinkmann et al. 1995b). In animal cells it appears to have more than one function: one in apoptosis and another in cell proliferation. We originally isolated *CAS* in a genetic screen for cDNAs that would render cancer cells resistant to bacterial toxins and immunotoxins (Brinkmann et al. 1995a). We found that a plasmid expressing *CAS* antisense cDNA rendered cells resistant against apoptosis induced by ADP-ribosylating toxins (*Pseudomonas* and diphtheria toxin) as well as by tumor necrosis factor (TNF). This suggests that *CAS* plays a role in apoptosis (Brink-

mann et al. 1995b). *CAS* also appears to have a function in cell proliferation. In yeast, the homologous gene *CSE1* is involved in chromosome segregation (Xiao et al. 1993) and is also necessary for B-type cyclin degradation in mitosis (Irninger et al. 1995). Direct experimental evidence for a role of *CAS* in the proliferation of human cells was a correlation of *CAS* expression with cell proliferation. *CAS* is preferentially expressed in tissues containing proliferating cells (testis or fetal liver), in rapidly growing tumor cell lines, and in fibroblasts that are stimulated to grow by serum addition (Brinkmann et al. 1995b).

The association of *CAS* with cell proliferation as well as apoptosis raises the possibility that *CAS* might be a cancer-related gene. Several genes that control cell growth and apoptosis, like *p53*, *bcl-2*,

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Figure 1 CAS MAPS on chromosome 20. Southern blots of *Pst*I-digested human genomic male and female, mouse, and hamster DNA and genomic DNA from somatic cell hybrids containing single human chromosomes were hybridized with a CAS cDNA probe. The mouse (m) or hamster (h) background of each line is indicated next to the number of the human chromosome. The CAS cDNA probe hybridizes under stringent conditions to mouse but not to hamster DNA, to human female and male DNA, and to human chromosome 20. The weak signal in the band of chromosome 12 is from a previous (unrelated) hybridization that was not completely stripped of the filter. There is no indication of a gene with homology to CAS on chromosome 12.

cyclin D, and *myc* (Tsujiimoto et al. 1985; Lowe et al. 1993; Hartwell and Kastan 1994; Hermeking and Eick 1994), play a role in cancer if they are mutated or if their expression is altered by genomic rearrangements. Another hint for a possible involvement of CAS in cancer is the function of the yeast homolog gene *CSE1*. Defects in *CSE1* lead to abnormal chromosome segregation in yeast. If the human homolog CAS gene were to have a similar function, alteration of CAS expression might cause abnormal chromosome segregation. Chromosome aneuploidies or aberrant chromosomes are observed frequently in cancer cells. Here we show that the CAS gene maps on

chromosome 20q13 close to a region that often contains amplifications associated with aggressive breast cancer. We also show that CAS is located on aberrant chromosomes in colon and breast cancer cell lines and that CAS is specifically amplified in BT-474 breast cancer cells.

RESULTS

CAS Maps on 20q13

The CAS gene was mapped to chromosome 20 by hybridization of genomic DNA from mouse or hamster somatic cell hybrids containing different human chromosomes with a CAS cDNA fragment (cDNA position 2100–2536, 1). Figure 1 shows that two *Pst*I fragments of human male and female genomic DNA hybridize with this probe. At least two hybridizing fragments were expected because the CAS probe contained an internal *Pst*I site. Both CAS fragments were detected in DNA of the mouse line that contains human chromosome 20.

For finer mapping, the human Centre d'Etude du Polymorphisme Humaine (CEPH) YAC Megabase library (Cohen et al. 1993) was screened by PCR with primers that amplify a 90-bp CAS-specific fragment from CAS cDNA and from human genomic DNA (see Methods). One yeast artificial chromosome (YAC) clone, 953-B-4, was positive with these primers. Microsatellite markers on and near YAC 953-b4 were identified and used to map the YAC relative to typed microsatellites (Polymeropoulos et al. 1993; Gyapay et al. 1994). YAC 953-B-4 overlaps with YACs containing the marker D20S176 on the long arm of human chromosome 20 indicating that the CAS gene lies within 2–3 Mb of that marker. This gene location, which was confirmed by fluorescent in situ hybridization (FISH; see below; Fig. 2), is close to the 20q13 region that is often amplified in breast, colon, and bladder cancer (Reznikoff et al. 1994; Tanner et al. 1994).

Southern Analyses of CAS Copy Number

The initial gene-mapping experiments established that CAS is linked to the amplification region in 20q13. However, CAS might be close to that region but not actually in it. Also, the 20q13

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Figure 2 Fluorescent in situ hybridization. Metaphase chromosomes of PBLs, breast cancer cells BT-474, MDA-MB-134, MDA-MB-361, and SW480 and COLO205 colon cancer cells were hybridized with digoxigenin-labeled P1/CAS and biotin-labeled control probes P1/2672 and P1/2567 (close to CAS on 20q) or P1/5461 (20p). (A) PBLs; (B) BT-474; (C) MDA-MB-134; (D) MDA-MB-361 with P1/CAS and P1/2672; (E) SW480; (F) COLO205 cells with P1/CAS and P1/5461 (20p). P1/CAS is visualized by fluorescein-anti-digoxigenin antibody (green) and controls with Texas Red-avidin (red). COLO201 colon, UACC812, and MDA-MB-157 breast cancer and CEM leukemia cells were also analyzed by FISH and had extra 20q marker chromosomes or additional copies of whole chromosome 20 (not shown; see Table 1).

region is, at least in breast cancer, not a homogeneous amplicon but consists of different regions that lie close together and contain various

degrees of possibly independent amplifications (Tanner et al. 1994). To test whether the CAS gene itself is amplified, we screened genomic DNA of cell lines from a variety of cancers (breast, colon, bladder, gastric, ovary, prostate, melanoma, leukemia, and lymphoma, and others; Table 1) by Southern hybridization. We compared the strength of the CAS signal to that produced by two control probes: β -actin and proliferating cell nuclear antigen (PCNA). Then, the ratio of CAS-specific signals to control signals was compared to the signal ratios of the tumor cell lines. Table 1 lists the PhosphorImager quantitation of Southern hybridizations with CAS and control probes using DNA from various cancer cell lines. Most cell lines did not show increased hybridization signals with the CAS-specific probe. However, CAS signals were elevated in BT474 breast cancer cells and in the colon cancer cell lines SW480, COLO201, and COLO205. This observation is in agreement with previous observations of amplifications in the long arm of chromosome 20 in breast and colon cancers (Reznikoff et al. 1994; Tanner et al. 1994). In addition, we found elevated CAS signals in CEM leukemia cells (Table 1).

FISH with P1/CAS

More detailed information about CAS amplification in cancer cells was obtained by FISH (Thompson and Gray 1993). To do this, a P1 phage containing the CAS gene (P1/CAS) was obtained from a human P1 genomic library. P1/CAS contains most (and probably all) of the CAS gene because (1) PCR primers for the 5' portion and 3' portion of the cDNA amplify 5' and 3' CAS gene fragments when using P1/CAS as template, (2) 5'- and 3'-end cDNA probes hybridize to P1/CAS, and (3) DNA sequencing reveals the presence of the CAS coding region in that phage (not shown). P1/CAS was labeled with digoxigenin and hybridized to inter-

Table 1. CAS Amplification in Cancer Cell Lines

Cell line		Southern signal elevation	FISH CAS copies	Chromosomes/ cell	Amplification	
PBLs	normal blood	N.D.	2	46	—	control
SK-BR3	breast cancer	—				
MCF-7	breast cancer	—				
MDA-MB-134	breast cancer	N.D.	4	86	<2-fold	normal chromosome; 20 + 20q isochromosome
MDA-MB-157	breast cancer	N.D.	2–6	54	<3-fold	large 20q marker chromosomes
UACC-812	breast cancer	N.D.	3	60	<2-fold	no normal 20, 20q marker chromosomes
MDA-MB-361	breast cancer	N.D.	3	56	<2-fold	aberrant chromosomes, CAS separated from control
BT-474	breast cancer	+	8–16	95–100	3-to 8-fold	no normal chromosome 20, CAS-specific amplification and translocations
COLO201	colon cancer	+	5	57–60	~2-fold	extra chromosome 20
COLO205	colon cancer	+	5	65–69	~2-fold	extra chromosome 20 + large 20q containing marker chromosomes
LS174T	colon cancer	—				
SW480	colon cancer	+	4–6	48–55	~2-fold	aberrant chromosomes with large 20q amplification (20q marker chromosomes)
K562	leukemia	—				
HL60	leukemia	—				
KG1	leukemia	—				
CEM	leukemia	+	6–8	83–86	~2-fold	extra chromosome 20

Southern blots with *EcoRI* cut-genomic DNA were hybridized with ³²P-labeled CAS and actin probes and the signals quantitated as described in Methods. The number of CAS signals by FISH on inter- and metaphase chromosomes (Fig. 2) reflect absolute numbers of CAS per cell, and the number of control signals with centromeric control P1/2672 or the 20p control P1/5461, the cell ploidy. Chromosome number/cell is chromosomes observed by FISH and agrees with published data (Hay et al. 1992), except for CEM, which should contain 45–47 chromosomes but we find 83–86 chromosomes. BT-474 cells contain many marker chromosomes; thus, the number of chromosomes do not reflect true ploidy. In addition, we evaluated several lymphomas (K562, CA46, Raji, Daudi), melanomas (G361, HTB63, FemX, A325, Molt4), ovarian cancer (OVCAR2, OVCAR3, CRL1874), gastric cancer (CRL1739, N87, HTG1, HTB-103), prostate cancer (LNCaP, DU145, PC3), bladder cancer (HTB2, HTB3, T24), and others (A431, HeLa, epidermoid; Huh7, Hep3B, liver; HTB79, HTB80, pancreas; U245, T98G, glioblastoma) by Southern blots without finding indication of CAS amplification in these cells.

and metaphase chromosomes and detected with fluorescein-labeled anti-digoxigenin antibodies. As reference probes for the determination of the relative amplification status of CAS, we used the following biotin-labeled P1 clones: P1/2672,

which hybridizes on 20q, centromeric to the position of the CAS gene in 20q13; P1/2567, which is telomeric to CAS; and P1/5461, which maps to the p arm of chromosome 20. The controls were visualized with Texas Red-avidin.

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Normal Cells

Figure 2 shows FISH with P1/CAS (green) and P1/2672 or P1/5461 (red) on metaphase chromosomes from peripheral blood lymphocytes (PBLs). The PBLs show two copies with each probe on the long arm of chromosome 20. Metaphase chromosomes show a linear orientation. P1/2672 is centromeric and P1/2567 (not shown) is telomeric to P1/CAS. This confirms the position of P1/CAS on 20q13 as defined by YAC mapping.

Breast Cancer Cells

We then analyzed five breast cancer cell lines: BT-474, MDA-MB-157, UACC-812, MDA-MB-361, and MDA-MB-134. BT-474 was chosen because Southern analyses indicated potential CAS amplification (Table 1). The others were evaluated because 20q13 amplifications occur frequently in breast cancer and because such amplifications were already demonstrated by comparative genomic hybridization (CGH) and FISH with other probes in UACC-812, MDA-MB-157, and BT-474 (Tanner et al. 1994). On BT-474 cells (Fig. 2), pairwise hybridization showed 8–16 signals with P1/CAS but only two signals per cell with the centromeric control probe. The two centromeric signals are linked to P1/CAS in a chromosome that appears to be the remainder of chromosome 20. Thus, relative to the region centromeric of CAS, as defined by the control phage (P1/2672), the CAS gene is amplified four- to eightfold. Pair-wise FISH on BT-474 cells with P1/CAS and P1/2567 as a control that hybridizes telomeric of CAS showed 8–12 signals (in another experiment up to 18) with CAS and 4 signals with P1/2567. Two of the four control signals were linked to CAS; the others were found on another chromosome separate from CAS. BT-474 cells do not contain a normal chromosome 20; all three probes hybridized to morphologically abnormal chromosomes. The amplified CAS gene is translocated to different abnormal chromosomes, each containing one to five copies of CAS. No double minute chromosomes were seen in BT-474 cells.

The other breast cancer cell lines that we analyzed, MDA-MB-134, -MB-157, -MB-361, and UACC-812, contained three to six copies of CAS, mainly present on abnormal marker chromosomes. MDA-MB-361 contains two normal copies of chromosome 20 (with CAS) and one addi-

tional copy of CAS separated from the telomeric 20q control on an aberrant chromosome. These cells also harbor a third copy of the control gene separated from CAS on another abnormal chromosome. MDA-MB-134 cells contain four to five copies of CAS, two to three normal copies of chromosome 20, and two CAS genes on a 20q isochromosome. UACC-812 and MDA-MB-157 contain three to six copies of CAS as well as of the centromeric control. Both signals remain linked and are found on abnormal chromosomes. UACC-812 cells do not contain a normal chromosome 20. In addition, we observed that the centromeric control probe is amplified to a higher degree than CAS (to five to eight copies). This is consistent with the observation of a second amplification “peak” centromeric from CAS in this cell line (Tanner et al. 1994).

Colon Cancer and Leukemia Cells

Three colon cancer cell lines (SW480, COLO201, COLO205) were identified as amplification candidates by Southern analyses (Table 1) and therefore analyzed by FISH with P1/CAS, the control probes described previously, and an additional 20p probe (P1/5461). We found that similar to MDA-MB-157 and UACC-812 cells and in contrast to BT-474 cells, CAS is located on aberrant chromosomes containing parts of 20q in SW480 and COLO205. Extra CAS copies are linked to 20q controls but separated from 20p (Fig. 2). The elevated copy number that is apparent in Southern blots of COLO201 (colon) and CEM (leukemia) cells (Table 1) is attributable to extras copies of chromosome 20. We found five copies of chromosome 20 per cell in COLO201 cells and six to eight copies per cell in CEM.

DISCUSSION

We have mapped the human CAS gene, which is associated with cell proliferation and apoptosis (Brinkmann et al. 1995b), on the long arm of chromosome 20 (q13). Southern hybridizations and FISH analyses show that CAS is specifically amplified four- to eightfold in BT-474 breast cancer cells. In addition, the CAS gene has spread in these cells to several abnormal chromosomes, each containing one to five copies of CAS. The copy number of CAS is also elevated in MDA-MB-361 and MDA-MB-157 breast cancer cell lines, SW480, COLO201 and COLO205 colon cancer

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Figure 3 Localization of CAS on 20q13. The localization of CAS relative to other markers and amplification regions on 20q13 is shown on an ideogram that was adapted from Tanner et al. (1994) with permission of O. Kallioniemi. (GHRH) growth hormone-releasing factor; (RPN2) riboprotein 2; (SRC) *src* oncogene; (TOP1) topoisomerase 1; (ADA) adenosine deaminase; (SEMG1) seminogelin 1; (PTPN1) protein tyrosine phosphatase; (MC3R) melanocortin-3 receptor; (RMC20C001) amplification probe defined by Tanner et al. (1994); (PCK1) PEP carboxykinase; (GNAS) guanosine NT-binding protein; (CHRNA4) cholerigic receptor α -4; (EDN3) endothelin 3; (ZNF8) zinc finger protein 8.

cells lines, and CEM leukemia cells. But in these cells (except for MDA-MB-361), they are part of large 20q aberrations (marker chromosomes) or extra copies of chromosome 20.

Amplification of the 20q13 region in which the human CAS gene is located correlates with aggressive breast cancer (Kallioniemi et al. 1994; Tanner et al. 1994). Amplifications in 20q are also associated with immortalization and genomic instability of uroepithelial cells transformed with the human papilloma virus 16 E6 gene, and 20q amplifications have been reported to be present also in colon and bladder cancer (Reznikoff et al. 1994). Inspection of the karyotypes of cancer cell lines indicates that part or all of chromosome 20 is overrepresented relative to other chromosomes in many cancer cell lines. These are not only of breast, colon, and bladder origin but also in lung cancer, gliomas, and mela-

nomas (Hay et al. 1992). The correlation of 20q (particularly q13) amplification with an aggressive type of breast cancer had led to the hypothesis that this region probably contains one or more novel oncogenes whose amplification may cause an aggressive cancer phenotype (Tanner et al. 1994).

CAS maps on 20q13 indicating that it is located close to the same amplification region that is described for cell lines and primary tumors (Tanner et al. 1994). The CAS gene is amplified in BT-474 breast cancer cells (which carry 20q13 amplifications) to a lesser degree than the maximally amplified 20q13 (Flpter 0.825) region in these cells. CAS, on Flpter 0.76 (A. Kallioniemi, and O. Kallioniemi, pers. comm.; see Fig. 3) is amplified four- to eightfold without being linked to markers ~2 Mb centromeric and telomeric from CAS. CAS is amplified to a higher degree than those markers. Flpter 0.825 is telomeric of CAS and of the control and is again amplified >10-fold (Tanner et al. 1994; RMC20C001 in Fig. 3). This shows that the 20q13 amplification in BT-474 cells consists not of a homogenous amplicon, but of different regions that lie close together, with various independent amplifications. The observation of the separation of the site of hybridization of P1/CAS and P1/2726 in BT-474 cells as well as in the MDA-MB-361 breast cancer cell line suggests that the q13 region of chromosome 20 may often be unstable in breast cancer cells. In BT-474 cells the amplified CAS gene is present on various abnormal chromosomes in different (one to five) copy numbers. It appears as if CAS has spread to other chromosomes by independent translocations or possibly transposon-like duplications of the CAS-containing 20q region. The latter possibility might explain how the CAS gene integrates into other chromosomes, whereas nearby DNA regions on either side of CAS do not follow the distribution of CAS. Alternatively, the CAS gene could have been present on double minutes and then integrated into another chromosome, but no double minutes were detected in BT-474 cells.

So far, known candidate genes mapping on 20q (among them, e.g., protein tyrosine phosphatase, zinc finger protein 8, or the *Src* oncogene) were excluded as being the amplified genes correlating with aggressive cancer phenotype (Tanner et al. 1994). This indicates that one or more new oncogenes lie in this region. The reasons to believe that CAS may be one of the genes in this region that are associated with cancer are

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(1) CAS is associated with cell proliferation and apoptosis, a phenotype that resembles that of genes like *myc* (although CAS is not homologous to *myc*), and (2) CAS may, like the yeast homolog *CSE1* gene, be involved in segregation of chromosomes in mitosis. Further analyses are required to evaluate the possibility that the CAS gene participates in the development or progression of some human cancers.

METHODS

Southern Analyses

Genomic DNAs from cancer cell lines and Southern blots were prepared by standard techniques (Sambrook et al. 1989). The CAS probe, a 436-bp *Asp700-XbaI* cDNA insert fragment from pCDM/HE17 (Brinkmann et al. 1995a,b) random prime-labeled with ^{32}P to $\sim 10^9$ cpm/ μg was hybridized for 15 hr at 50°C in 50% formamide to a somatic cell hybrid blot (ONCOR) or to Southern blots containing *EcoRI*-cut genomic DNA from cancer cell lines. The signals from cell line blots hybridized with CAS and control probes were quantitated on a Molecular Dynamics PhosphorImager. Variability in the amount of blotted DNA was compensated by determining the ratio between CAS and control signals that was set to 1 for human placenta DNA. Other samples were calculated relative to that. We used actin (on chromosome 7) as control probe. Chromosome 7 is overrepresented in some cancer cells. Therefore, the use of actin as control for comparisons to signals from genes on other chromosomes will result in conservative copy number estimation, and increases relative to actin correspond likely to gene copy number increase. We used a PCNA probe as additional 20p control.

Isolation of CAS Gene Containing YAC and P1 Clones

A6 YAC containing the CAS gene was identified by PCR screening (Polymeropoulos et al. 1993) with CAS-specific primers 5'-GACATCCCGTCTTCCTATATG-3' (forward) and 5'-AAGAAGCCTCACTAGAGCAGGA-3' (reverse). The program "yacs" (M.H. Polymeropoulos, unpubl.) was used to locate microsatellite markers on or near the obtained YAC address and position it relative to known markers. A P1 phage containing the CAS gene (P1/CAS) was obtained from a human P1 genomic library by PCR screening (Genome Systems, St. Louis, MO) with the same primers that we used for the YAC screen.

FISH

Interphase and metaphase chromosomes were hybridized with digoxigenin-dUTP-labeled P1/CAS and biotin-dUTP-labeled control probes P1/2672 and P1/2567 (close to CAS on 20q) or P1/5461 (20p). P1/CAS was visualized by fluorescein-anti-digoxigenin antibody (green) and the controls with Texas Red-avidin (red), using a triple band pass

filter set and DAPI counterstain. FISH analyses were performed by M. Valentine at Genome Systems. The control clones P1/2672 and P1/2567 are from Genome Systems and were mapped previously (M. Valentine, pers. comm.) ~ 2 Mb centromeric (2672) and telomeric (2567) from CAS on chromosome 20 or on 20p (P1/5461).

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