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CAS, the Human Homologue of the Yeast Chromosome-Segregation Gene *CSE1,* in Proliferation, Apoptosis, and Cancer

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Proliferation—that is, progression through the cell cycle—and apoptosis are opposing cellular mechanisms, one leading to multiplication and the other to elimination of cells. Although proliferation and apoptosis can function independently of each other, the two pathways are often linked in mammalian cells. For example, cell-cycle checkpoints control the initiation, progression, and completion of the cycle, giving cells a chance to correct mistakes before continuing with subsequent steps of the cycle. However, if the mistakes are not correctable, the cells are eliminated by execution of apoptosis. The integration of these two seemingly conflicting pathways is a useful strategy in multicellular organisms, in which cells can be replaced readily but in which the proliferation of potentially tumorigenic cells must be suppressed.

Because of the link between cell-cycle and apoptotic pathways, it is not surprising that proteins that play a role in both mechanisms are associated with, and may cause, cancer. One well-known example is the *P53* gene, which acts in the G1 checkpoint of the cell cycle and is also necessary for apoptosis (Liebermann et al. 1995; Agarwal et al. 1998). Many cancers, inherited or sporadic, involve loss of p53 function. Another example is the *MYC* oncogene, which stimulates proliferation at the G1→S transition in the cell cycle and simultaneously causes cells to be very sensitive to apoptosis (Evan et al. 1992).

Like p53 and Myc, the recently discovered cellular apoptosis–susceptibility (CAS) protein, the human homologue of the yeast chromosome-segregation protein CSE1 (Xiao et al. 1993; Brinkmann et al. 1995*a,* 1995*b,* 1996*a,* 1996*b*), is associated with both apoptosis and cell proliferation and also appears to play a role in cancer. Interestingly, unlike most well-known cancer genes, which function in the G1→S transition of the cell cycle, *CAS* is necessary in a mitotic checkpoint that may assure the accurate segregation of chromosomes.

A cell-cycle checkpoint that permits chromosome segregation only if all chromosomes are properly aligned has been established, in part, through direct micromanipulation of chromosomes in insect cells (Nicklas 1997). However, in contrast to the vast amount of knowledge available regarding the G1→S checkpoint and the role of oncogenes in overriding it, knowledge of the molecular basis of the mitotic checkpoint is only now emerging. This checkpoint is probably crucial in the development of human cancer: Genomic instability is one hallmark of cancer, and a major mechanism by which the genome of cancer cells becomes unstable may be the overriding of cell-cycle checkpoints that are responsible for accurate chromosome maintenance. In this article, I discuss the evidence that the human *CAS* gene, like the yeast chromosome-segregation gene *CSE1*, is involved directly or indirectly in the spindle checkpoint. The fact that CAS is also associated with apoptosis suggests that it could be a safeguard against genomic instability, participating in apoptosis when correct chromosome segregation cannot be achieved.

CAS in Apoptosis and Proliferation

The *CAS* gene was originally identified in a genetic screen for genes that affect the sensitivity of breast cancer cells toward toxins and immunotoxins that are used in experimental cancer therapy (Brinkmann et al. 1995*a,* 1995*b,* 1996*a*). Using an expression-selection cloning approach, we sought plasmids that would render breast cancer cells resistant to immunotoxins. Several of the identified plasmids contained CAS antisense cDNA fragments, suggesting that the CAS protein participates in immuntoxin-dependent cytotoxicity. The attenuation of CAS protein, by expression of antisense cDNA, reduced the sensitivity of the cells to apoptosis induced by immunotoxins and various bacterial toxins (Brinkmann et al. 1996*a*) or by tumor-necrosis factor (TNF)–α or TNF-

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β (fig. 1). Recent observations have indicated that the apoptotic pathway that is induced by bacterial toxins depends on the caspase CPP32 (A. Hafkemeyer, U. Brinkmann, and I. Pastan, unpublished data), and at least some of the steps in this pathway also occur during TNF-α–induced apoptosis. Since CAS depletion was shown to prevent apoptosis, we concluded that *CAS* expression is necessary for apoptosis.

CAS is highly homologous to yeast *CSE1,* an essential gene that is necessary for accurate chromosome segregation in mitosis (Xiao et al. 1993; Brinkmann et al. 1995*a*; Irniger et al. 1995). Conditionally lethal CSE1 mutants show a chromosome-segregation–defective phenotype at the nonpermissive temperature, and they are unable to degrade the cyclin B protein, in which proteolysis represents a key checkpoint in mitosis. Therefore, *CSE1*-mutant yeast cells become arrested in mitosis. Because the yeast genome is known completely and because no other yeast gene has a similarly high homology to *CAS, CSE1* can be considered the functional yeast counterpart of *CAS*. The high homology of *CAS* to this yeast cell-cycle gene and the observation that *CAS* is highly expressed in proliferating cells but is expressed only at low levels in most quiescent cells and tissues suggest that CAS acts not only in apoptosis but also in the cell cycle. Indeed, we found that CAS is required for the mitotic checkpoint in the mammalian cell cycle—that is, the same checkpoint where CSE1 acts in yeast (Ogryzko et al. 1997). As with *CSE1* mutations, CAS depletion causes accumulation of cyclin B in the cell-cycle–arrested cells. Conversely, overexpression of *CAS* is associated with rapid cell growth, malignant transformation, and possibly with defects in a mitotic checkpoint that assures accurate chromosome segregation.

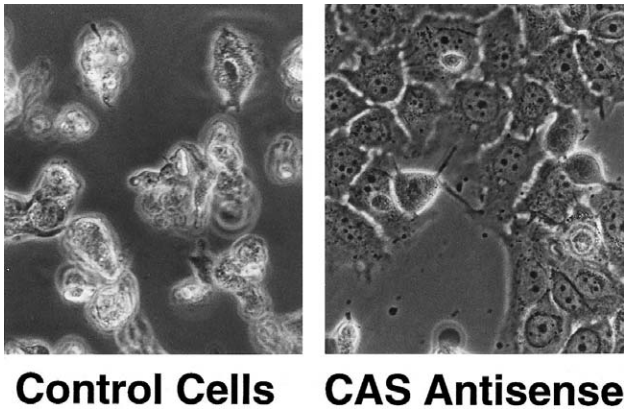

Figure 1 Role of CAS in apoptosis. MCF-7 breast cancer cells were transfected with a CAS-antisense plasmid or with control plasmids and then were exposed to 1 μg TNF-α/ml for 24 h. Antisense-mediated depletion of CAS protein rendered the cells less sensitive to TNF-induced apoptosis. In a similar manner, cells also become resistant to apoptosis induced by *Pseudomonas* exotoxin A and by diphtheria toxin.

The Molecular Function and Regulation of CAS

One mechanism by which CAS affects proliferation as well as apoptosis is its function as a nuclear-transport factor. CAS mediates nuclear-to-cytosolic recycling of importin-α and possibly other factors that mediate the transport of specific proteins into the nucleus (Kutay et al. 1997; Ullman et al. 1997). Some proteins that enter the nucleus by the importin pathway are necessary for the execution of and/or progression through mitosis—for example, cyclin-dependent kinases (CDKs) and cyclin/CDK complexes, kinases, p53, and transcription factors (retinoblastoma gene product and nucleus factor κB) (Gallant et al. 1995; Thomas et al. 1996; Middeler et al. 1997). This explains the observation that depletion of CAS protein causes a cell-cycle arrest in the G2 phase. Nuclear transport also appears to be important for apoptosis. For example, transcription factors, p53, and probably nucleases need to be transported into the nucleus during apoptosis. Therefore, the involvement of the CAS protein in apoptosis can be explained by the requirement of CAS for nuclear transport of proteins that are necessary for induction and/or execution of apoptosis.

How is the dual function of CAS in proliferation and apoptosis regulated? Central regulatory pathways, such as the mitogen-activated protein (MAP)-kinase cascade, regulate proliferation as well as apoptosis (Seger and Krebs 1995). For example, the well-known oncoprotein Myc, which is a player in the MAP-kinase pathway, stimulates cell proliferation and simultaneously sensitizes cells to apoptosis (Evan et al. 1992). CAS is also linked to the MAP-kinase cascade because it contains, at its N-terminus, a phosphorylation site for MEK, a kinase that acts upstream of the MAP kinase, in this pathway. It is likely that the function of CAS is regulated by phosphorylation. Recent data indicate that MEK phosphorylation determines whether CAS localizes predominantly in the cytoplasm or in the nucleus. In turn, this control of intracellular localization would directly affect the nuclear-transport function of CAS and, hence, indirectly affect that of the importin proteins.

Although the CAS protein needs to be in the nucleus to perform its nuclear-transport function, in most cells, the majority of CAS protein is found in the cytoplasm, where it is associated with microtubules (Scherf et al. 1996). The association of CAS with cytoplasmic microtubules may create a reservoir of inactive CAS protein; alternatively, microtubule-associated CAS may have distinct biological functions during interphase. In meiosis and mitosis, CAS is strongly associated with centrioles and with the mitotic spindle. It is particularly interesting

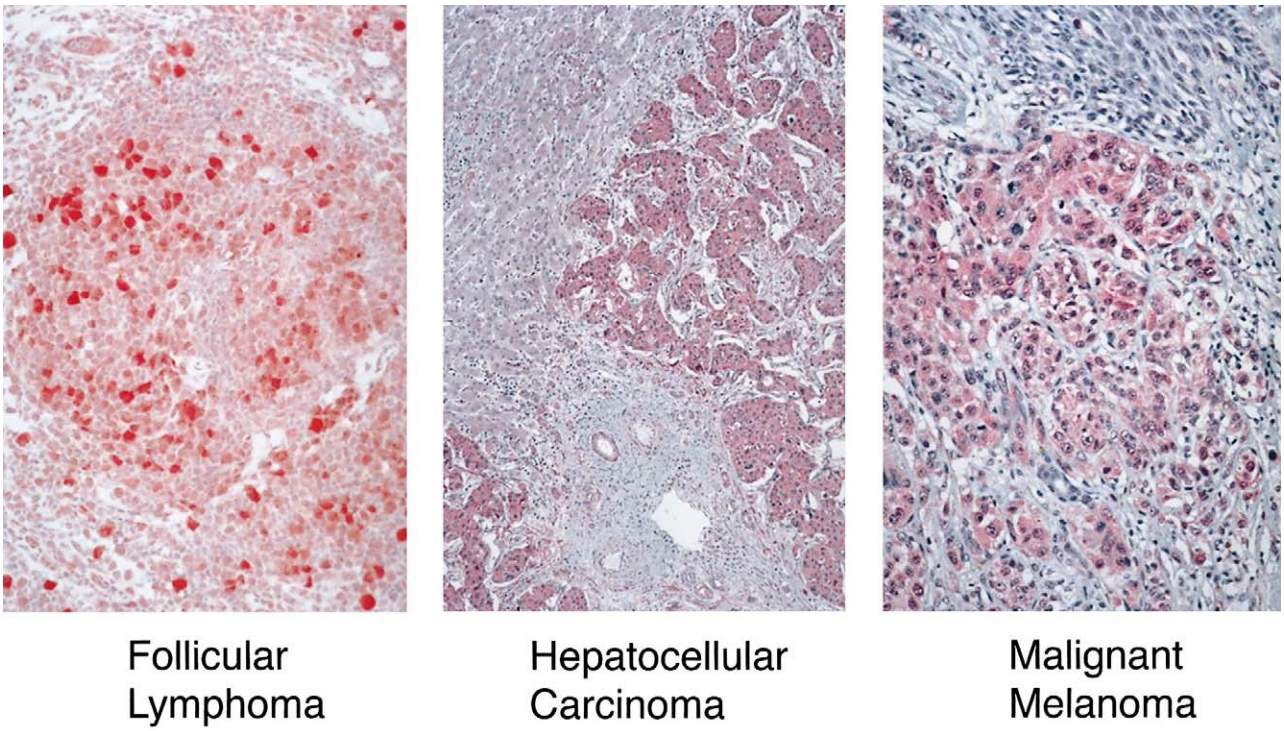

Figure 2 Immunohistological detection of CAS protein in human tumors. Immunohistochemistry with anti-CAS antibodies, which stained brown or red, shows that CAS protein is abundant in the malignant cells of various tumors, including leukemias (e.g., follicular lymphoma; *left*), hepatocarcinoma (*middle*), and malignant melanoma (*right*).

that MEK, which phosphorylates CAS and controls its intracellular localization, also localizes to the spindle and is involved in a checkpoint that permits mitosis only if all chromosomes are properly aligned (Nicklas 1997). The observation of chromosome-segregation defects in yeast cells that lack *CSE1* function (Xiao et al. 1993) strengthens the possibility that human CAS participates in a mitotic checkpoint that assures proper chromosome segregation.

CAS and Cancer

CAS is located on human chromosome 20q13 (Brinkmann et al. 1996*b*), a region with a remarkable degree of instability in various tumors. Amplifications of 20q13, consisting of several independent amplicons, with *CAS* present in one of them (Tanner et al. 1994), are observed frequently in aggressive types of breast cancer. *CAS* and 20q13 amplifications also are frequent in colon cancer (Ried et al. 1996) and in transformed uroepithelial cells, in which these amplifications correlate with, and may be causative for, a high degree of genetic instability (Savelieva et al. 1997).

CAS expression (mRNA and protein) appears to be generally upregulated in a variety of cancers, not only in tumors that contain amplifications of the *CAS* gene. Immunohistochemistry with anti-CAS antibodies confirms that the expression of CAS correlates with the development of cancer (fig. 2; also see Wellmann et al. 1997). A comparison of CAS expression in different disease stages demonstrates that increased levels of CAS coincide with disease progression, suggesting that the evaluation of CAS expression by immunohistochemistry or by PCR may be a useful diagnostic tool.

There are at least two mechanisms, which are not mutually exclusive, by which CAS could induce or advance tumorigenesis. First, overexpression of CAS (e.g., owing to gene amplification) may interfere with the strictly regulated nuclear transport of potential oncogenes or tumor suppressors. It is striking in this context that many cancer-associated proteins require highly regulated nuclear transport. Changes in the nuclear-transport pattern of proteins such as p53, the retinoblastoma protein, and BRCA1 (Thomas et al. 1996; Middeler et al. 1997; Wang et al. 1997) are associated with the development of cancer. Furthermore, in *Drosophila*, which can serve as a model for both normal developmental pathways and tumorigenesis (St. John and Xu 1997; Rodriguez et al. 1998 [in this issue]), the *pendulin* gene has been identified as a tumor suppressor; *pendulin* encodes the fly homologue of importin-α, a nuclear shut-

tling factor that interacts directly with CAS (Kussel and Frasch 1995). This strongly supports the hypothesis that interference with the nuclear-transport function of CAS may play a role in cancer. Second, as suggested by the chromosome-segregation phenotype of *CSE1* yeast cells, *CAS* depletion (and probably overexpression) may lead to the aneuploidy often seen in advanced tumor cells. In normal yeast and mammalian cells, the spindle checkpoint assesses the alignment of chromosomes on the spindle and allows execution of mitosis only when the correct distribution of chromosomes into the daughter cells is possible (Nicklas 1997). Cells with improperly aligned chromosomes are arrested until the chromosomes realign in a correct manner. If correct chromosome realignment is not possible, the arrested cells eventually die. Recent work with human cells confirms this view. Schaar et al. (1997) used antibodies or dominantly acting mutant proteins to block mitosis, by inhibiting the kinetochore-based motor protein, centromere protein-E. They found that chromosomes in treated cells formed but failed to localize to the spindle equator, often oscillating around it for many hours. Eventually, however, all these cells underwent sudden death by apoptosis, as is expected when the mitotic checkpoint is engaged and cannot be released.

Yeast, a single-celled eukaryote, lacks a mechanism for apoptosis, but other aspects of the cellular response to chromosomal misalignment are similar in yeast and in human cells. Yeast *CSE1* mutants show genetic instability, such as aberrant chromosome segregation in mitosis, and undergo arrest during cell division, much like CAS-depleted human cells (Xiao et al. 1993). In addition, genomic instability (e.g., in transformed uroepithelial cells) has been directly associated with amplification of the 20q13 region that contains the *CAS* gene. This chromosomal 20q13 region contains at least one gene that is involved in maintaining genome integrity, including the *CAS* candidate gene (Savelieva et al. 1997). Because CAS is associated with the mitotic spindle, it is possible that CAS is part of the cell-cycle checkpoint that assures that each daughter cell obtains a complete set of chromosomes. High constitutive expression of CAS might then interfere with or override that checkpoint. The fact that CAS is also associated with apoptosis suggests that it serves as a safeguard against the consequences of genomic instability, by rendering cells that might gain or lose chromosomes particularly prone to apoptosis.

Prospects

In addition to the insights CAS provides into connections between nuclear-protein transport, cell-cycle checkpoints, and apoptosis, one major point of interest is the connection of CAS with cancer and possibly with other diseases. To date, no inherited defects or aberrations of the CAS gene have been correlated with human diseases. Since CAS functions in apoptosis as well as in proliferation—two processes that act together to assure normal development—one would expect true null alleles of *CAS* to cause embryonic lethality. Indeed, the yeast homologue *CSE1* is an essential gene, and mutations in *pendulin,* an importin-α homologue that almost certainly acts in an equivalent nuclear-transport pathway in fly cells, not only displays aberrant cell proliferation and tumors but ultimately causes the death of *Drosophila* larvae (Kussel and Frasch 1995). *CAS* has yet to be identified in *Drosophila,* but this may prove to be a useful system for analysis of the consequences of weak or overexpressed alleles of this gene. *CAS* mutations, in humans or in flies, could interfere with regulated import of nuclear proteins and might lead to developmental abnormalities and to loss of fidelity in chromosomal disjunction, in somatic or germ-line cells.

Sporadic *CAS* aberrations, particularly amplifications of *CAS* and of the chromosome 20q13 region that harbors it, are observed frequently in human tumors, such as breast cancer and colon cancer, and amplifications of this region correlate with aggressive disease (Tanner et al. 1994; Brinkmann et al. 1996*b;* Ried et al. 1996; Savelieva et al. 1997). The observation of very high CAS-protein levels in other neoplasms, without gene amplification, and the fact that increased expression correlates with the progression of malignancy also suggest the possibility of mutations that upregulate the expression of *CAS*. The analysis of whether and how CAS contributes to the progression of cancer is the focus of our present research.

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References

Agarwal ML, Taylor WR, Chernov MX, Chernova OB, Stark GR (1998) The p53 network. J Biol Chem 273:1–4

Brinkmann U, Brinkmann E, Gallo M, Pastan I (1995*a*) Cloning and characterization of a cellular apoptosis susceptibility gene, the human homologue to the yeast chromosome segregation gene CSE1. Proc Natl Acad Sci USA 92: 10427–10431

Brinkmann U, Brinkmann E, Gallo M, Scherf U, Pastan I (1996*a*) Role of CAS, a human homologue to the yeast chromosome segregation gene CSE1, in toxin and TNF mediated apoptosis. Biochemistry 35:2891–2899

Brinkmann U, Brinkmann E, Pastan I (1995*b*) Expression cloning of cDNAs that render cancer cells resistant to Pseudo-

monas and diphtheria toxin and immunotoxins. Mol Med 1:206–216

Brinkmann U, Gallo M, Polymeropoulos MH, Pastan I (1996*b*) 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 cells. Genome Res 6:187–194

Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, et al (1992) Induction of apoptosis in fibroblasts by c-myc protein. Cell 69:119–128

Gallant P, Fry AM, Nigg EA (1995) Protein kinases in the control of mitosis: focus on nucleocytoplasmic trafficking. J Cell Sci 19:21–28

Irniger S, Piatti S, Michaelis C, Nasmyth K (1995) Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. Cell 81:269–278

Kussel P, Frasch M (1995) Pendulin, a *Drosophila* protein with cell cycle–dependent nuclear localization, is required for normal cell proliferation. J Cell Biol 129:1491–1450

Kutay U, Bischoff FR, Kostka S, Kraft R, Gorlich D (1997) Export of importin alpha from the nucleus is mediated by a specific nuclear transport factor. Cell 90:1061–1071

Liebermann DA, Hoffman B, Steinman RA (1995) Molecular controls of growth arrest and apoptosis: p53-dependent and independent pathways. Oncogene 11:199–210

Middeler G, Zerf K, Jenovai S, Thulig A, Tschodrich-Rotter M, Kubitscheck U, Peters R (1997) The tumor suppressor p53 is subject to both nuclear import and export, and both are fast, energy-dependent and lectin-inhibited. Oncogene 14:1407–1417

Nicklas RB (1997) How cells get the right chromosomes. Science 275:632–637

Ogryzko VV, Brinkmann E, Howard BH, Pastan I, Brinkmann U (1997) Antisense inhibition of CAS, the human homologue of the yeast chromosome segregation gene CSE1, interferes with mitosis in HeLa cells. Biochemistry 36: 9493–9500

Ried T, Knutzen R, Steinbeck R, Blegen H, Schrock E, Heselmeyer K, du Manoir S, et al (1996) Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. Genes Chromosom Cancer 15:234–245

Rodriguez A, Chen P, Abrams JM (1998) Molecular prophets of death in the fly. Am J Hum Genet 62:514–519 (in this issue)

Savelieva E, Belair CD, Newton MA, DeVries S, Gray JW, Waldman F, Reznikoff CA (1997) 20q Gain associates with immortalization: 20q13.2 amplification correlates with genome instability in human papillomavirus 16 E7 transformed human uroepithelial cells. Oncogene 14:551–560

Schaar BT, Chan GKT, Maddox P, Salmon ED, Yen TJ (1997) CENP-E function at kinetochores is essential for chromosome alignment. J Cell Biol 139:1373–1382

Scherf U, Pastan I, Willingham MC, Brinkmann U (1996) The human CAS protein, which is homologous to the CSE1 yeast chromosome segregation gene product, is associated with microtubules and mitotic spindle. Proc Natl Acad Sci USA 93:2670–2674

Seger R, Krebs EG (1995) The MAPK signaling cascade. FASEB J 9:726–735

St John MAR, Xu T (1997) Understanding human cancer in a fly? Am J Hum Genet 61:1006–1010

Tanner MM, Tirkkonen M, Kallioniemi A, Collins C, Stokke TY, Karhu R, Kowbel D, et al (1994) Increased copy number at 20q13 in breast cancer: defining the critical region and exclusion of candidate genes. Cancer Res 54:4257–4260

Thomas RC, Edwards MJ, Marks R (1996) Translocation of the retinoblastoma gene product during mitosis. Exp Cell Res 15:227–232

Ullman KS, Powers MA, Forbes DJ (1997) Nuclear export receptors: from importin to exportin. Cell 90:967–970

Wang H, Shao N, Ding QM, Cui J, Reddy ES, Rao VN (1997) BRCA1 proteins are transported to the nucleus in the absence of serum and splice variants BRCA1a, BRCA1b are tyrosine phosphoproteins that associate with E2F, cyclins and cyclin dependent kinases. Oncogene 15:143–157

Wellmann A, Krenacs L, Fest T, Scherf U, Pastan I, Raffeld M, Brinkmann U (1997) Localization of the cell proliferation and apoptosis associated CAS protein in lymphoid neoplasms. Am J Pathol 150:25–30

Xiao Z, McGrew JT, Schroeder AJ, Fitzgerald-Hayes M (1993) CSE1 and CSE2, two new genes required for accurate mitotic chromosome segregation in *Saccharomyces cerevisiae*. Mol Cell Biol 13:4691–4702