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KAI1, a Metastasis Suppressor Gene for Prostate Cancer on Human Chromosome 11p11.2

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A gene from human chromosome 11p11.2 was isolated and was shown to suppress metastasis when introduced into rat AT6.1 prostate cancer cells. Expression of this gene, designated *KAI1*, was reduced in human cell lines derived from metastatic prostate tumors. *KAI1* specifies a protein of 267 amino acids, with four hydrophobic and presumably transmembrane domains and one large extracellular hydrophilic domain with three potential N-glycosylation sites. *KAI1* is evolutionarily conserved, is expressed in many human tissues, and encodes a member of a structurally distinct family of leukocyte surface glycoproteins. Decreased expression of this gene may be involved in the malignant progression of prostate and other cancers.

Metastasis, the main cause of death for most cancer patients, remains one of the most important but least understood aspects of cancer (*1*). Both positive and negative regulators of metastasis are likely to exist. The existence of genes involved in metastasis suppression is suggested by somatic cell genetic studies in which nonmetastatic and metastatic tumor cells are hybridized and the resultant cell hybrids are tumorigenic but no longer metastatic (*2*). For example, the metastatic ability of rat AT6.1 prostate cancer cells was suppressed when they were fused to nonmetastatic cancer cells (*3*), and the putative metastasis suppressor gene was mapped to human chromosome 11p11.2-13 (the p11.2-13 region of chromosome 11) by microcell-mediated chromosome transfer (*4*).

To clone this metastasis suppressor gene on human chromosome 11, we isolated genomic DNA fragments from the p11.2-13 region by human-specific Alu element–mediated polymerase chain reaction (Alu-PCR) (*5*) with DNAs from the metastasis-suppressed microcell hybrid AT6.1-11-1 and from the nonsuppressed hybrids AT6.1-11-2 and AT6.1-11-3 (*4*, *6*). The Alu-PCR fragments in the AT6.1-11-1 DNA were then used as probes to screen a complementary DNA (cDNA) library from the suppressed cell hybrid clone AT6.1-11-1 that contained human chromosomal region 11cen-p13. Of five cDNA clones obtained (*7*), all were expressed in the suppressed hybrid but not in the nonsuppressed hybrids, as detected by reverse transcription PCR (RT-PCR). Northern (RNA) blot analysis (*8*) of human prostate tissue and the metastasis-suppressed hybrid cells (AT6.1-11-1) revealed that two of the cDNA clones detected 2.4-kb and 4.0-kb sequences in human tissue and in the suppressed hybrid cells, respectively. These two sequences were not detected in the parental AT6.1 cells or in the nonsuppressed hybrid cells (AT6.1-11-2 and AT6.1-11-3). The clone that detected the 2.4-kb sequence, designated *KAI1* for *kang ai* (Chinese for anticancer), was abundant in AT6.1-11-1 (Fig. 1A) and was analyzed further.

Southern (DNA) blot analysis confirmed that *KAI1* was isolated from human chromosome 11p11.2-13. Only the cell hybrids containing the p11.2-13 region involved in metastasis suppression exhibited the pattern observed with normal human DNA when hybridized to the *KAI1* probe. Fluorescence in situ hybridization of a *KAI1* probe to metaphase chromosomes further localized *KAI1* to the p11.2 region (*9*).

The *KAI1* cDNA has a single open reading frame (nucleotide positions 166 to 966) that predicts a protein of 267 amino acids with a molecular mass of 29,610 daltons (Fig. 1B). An Alu element is present in the 3′-untranslated region of the cDNA. The predicted protein has four hydrophobic and presumably transmembrane domains and one large extracellular hydrophilic domain with three potential N-glycosylation sites. A search of the GenBank and European Molecular Biology Laboratory (EMBL) databases revealed that *KAI1* is identical to three cDNA clones from human lymphocytes, designated C33, R2, and IA4 by different laboratories (*10*–*12*). C33 is associated with the inhibition of virus-induced syncytium formation (*10*), R2 is strongly up-regulated in mitogen-activated human T cells (*11*), and IA4 is expressed in large amounts in several B lymphocyte lines (*12*). One of these studies showed that the protein is localized to the cellular membrane and is highly glycosylated (*10*), which is consistent with the predicted molecular features.

To investigate whether *KAI1* was responsible for the metastasis suppression in AT6.1-11-1, we subcloned *KAI1* cDNA into a constitutive expression vector (*13*) and transfected it into parental AT6.1 cells. Individual transfectants were analyzed for *KAI1* expression and for their ability to suppress lung metastases of AT6.1 cells in nude mice (*14*). The vector alone was also transfected as a negative control. Expression of *KAI1* resulted in a significant suppression of the number of lung metastases per mouse, but it did not affect the growth rate of the primary tumor. These findings were observed in different experiments with multiple clones; one such experiment is shown in Table 1. Although the parental AT6.1 cells yielded 58 metastases per mouse, two transfectants that expressed large amounts of *KAI1* mRNA (similar to those for AT6.1-11-1) yielded only 6 or 7 metastases per mouse. Three vector control transfectants produced 30 to 47 metastases per mouse. Northern blot analysis showed that *KAI1* expression was undetectable or very low in 28 lung metastases from the *KAI1* transfectants, suggesting that selection for cells with absent or reduced *KAI1* expression resulted in metastasis formation. In addition, *KAI1* expression was reduced in human cell lines derived from metastatic prostate tumors (*15*) as compared with its expression in normal prostate tissue (Fig. 2); this finding suggests a possible suppressive role for *KAI1* in the metastasis of human prostate cancer.

To rule out the possibility that metastasis suppression by *KAI1* is the result of an indirect immune mechanism, we performed

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Table 1. Suppression of metastasis by introduction of *KAI1* into AT6.1 rat prostate cancer cells (*14*). Data are from a large age-matched cohort of "side-by-side" nude mice into which cells were inoculated at the same time. *KAI1* expression was determined by Northern blot analysis. The *KAI1* signals on the Northern blot were scored by a densitometer; the value for AT6.1KAI-1 was standardized to 10 and the values for the other clones were adjusted accordingly. Latency is the time from injection to the appearance of a palpable tumor. *P* values are for comparisons of *KAI1*-expressing clones and their control clones.

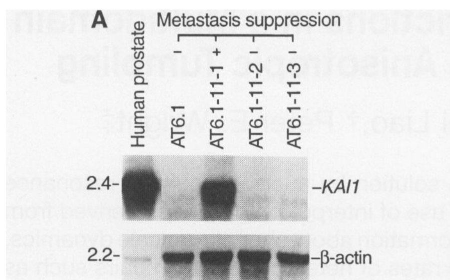
Transfectant	*KAI1* mRNA expression	Latency (days)	Tumor age (days)	Tumor weight (g) at excision	Number of mice inoculated	Number of mice with metastases	Mean number of metastases per mouse (range)	*P*
AT6.1	0	4.3	27	2.58	19	19	58 (32–135)	
AT6.1-11-1	10	3.7	37	2.79	7	6	7 (0–9)	<0.005*
AT6.1-11-2	0	4.2	37	2.78	6	6	26 (20–40)	
AT6.1VEC-1	0	4.9	43	2.32	17	17	30 (16–57)	
AT6.1VEC-2	0	4.0	43	3.26	17	17	30 (12–71)	
AT6.1VEC-3	0	5.5	43	2.57	18	18	47 (15–183)	
AT6.1KAI-1	10	4.2	43	3.99	20	18	6 (0–14)	<0.001†
AT6.1KAI-2	7	4.5	41	1.79	19	17	7 (0–17)	<0.001†
AT6.1KAI-3	1	4.5	43	2.56	19	18	23 (0–36)	<0.02†

*Compared to the number of metastases with AT6.1-11-2 cells. †Compared to the mean number of metastases with all three vector transfectants.

two types of experiments. First, the metastasis-suppressed clones were inoculated into mice with severe combined immunodeficiency disease (SCID), which have immune systems that are more compromised than those of nude mice. These studies demonstrated that metastasis suppression occurred even in SCID mice (*16*). Second, the *KAI1* gene was introduced into highly metastatic rat mammary cancer cells by means of microcell-mediated chromosome transfer. These cells retained their ability to metastasize (*17*), even though the hybrid expressed certain amounts of *KAI1* mRNA (*18*). These data indicate that a more direct mechanism appears to be responsible for metastasis suppression by *KAI1*. Consistent with this possibility, the invasive ability of AT6.1-11-1 hybrid cells expressing large amounts of *KAI1* was about half that of parental AT6.1 cells or nonsuppressed AT6.1-11-2 hybrid cells, as measured by Boyden chamber invasion assays (*19*).

To evaluate the amount of expression of *KAI1* in various human tissues, we performed Northern blot analyses. The 2.4-kb *KAI1* transcript was detected in all the human tissues tested. It was most abundant in the prostate, lung, liver, kidney, bone marrow, and placenta; moderately abundant in the mammary gland, pancreas, skeletal muscle, and thymus (*20*); and least abundant in the brain, heart, ovary, stomach, and uterus (Fig. 3). Southern blot analyses showed that the *KAI1* coding sequence is highly conserved in human, monkey, dog, and rabbit and is moderately conserved in cow, rat, and mouse (*20*). The evolutionary conservation and wide tissue distribution of *KAI1* suggest that this gene has an essential biological function.

KAI1 belongs to a structurally distinct family of membrane glycoproteins (*21*) that includes ME491/CD63 (*22*), MRP-1/CD9 (*23*), TAPA-1 (*24*), CD37 (*25*), and CD53 (*26*), most of which have been identified as leukocyte surface proteins. These proteins all have four transmembrane domains and a large extracellular N-glycosylated domain. Although the biological functions of these proteins are mostly unknown, their membrane localization and extensive glycosylation suggest that they function in cell-cell interactions and cell–extracellular matrix interactions (*27*), both of which are important in invasion and metastasis. In particular, the N-glycosylation of these molecules is consistent with their presumed role in metastasis suppression because the association between processing of N-linked oligosaccharides and metastatic phenotype is well documented (*28*). In addition to *KAI1* expression, the expression of two other members of this family has been correlated with metastasis. The *MRP-1* gene product is



B

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1 MGSACIKVTK YFLFLFNLIF FILGAVILGF GVWILADKSS FISVLQTSSS
51 SLRMGAYVFI GVGAVTMLMG FLGCIGAVNE VRCLLGLYFA FLLLILIAQV
101 TAGALFYFNM GKLKQEMGGI VTELIRDYNS SREDSLQDAW DYVQAQVKCC
151 GWVSFYNWTD NAELMNRPEV TYPCSCEVKG EEDNSLSVRK GFCEAPGNRT
201 QSGNHPEDWP VYQEGCMEKV QAWLQENLGI ILGVGVGVAI IELLGMVLSI
251 CLCRHVHSED YSKVPKY
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Fig. 1. Cloning and characterization of *KAI1* cDNA (*7*). (**A**) Northern blot of mRNA from normal human prostate tissue and from metastatic and nonmetastatic tumor cells hybridized to the *KAI1* probe (*8*). Metastasis suppression (+) or nonsuppression (−) is indicated for AT6.1 parental and hybrid cells (*4, 6*). Polyadenylated RNA (2 μg) was loaded in each lane. A probe for rat β-actin was used as a control (to demonstrate that approximately equal amounts of RNA were loaded in each lane). Nucleotide sizes are indicated in kilobases. (**B**) Deduced amino acid sequence of *KAI1* (*29*). The four putative transmembrane domains are underlined, and the potential N-linked glycosylation sites are doubly underlined. The cDNA sequence has been deposited in GenBank (accession number U20770).

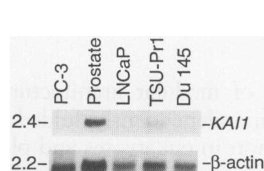

Fig. 2. Expression of *KAI1* in normal human prostate tissue and in cell lines PC-3, LNCaP, TSU-Pr1, and Du 145 derived from metastatic prostate cancers (*17*). The blot had 15 μg of total RNA in each lane and was hybridized to the *KAI1* probe (*8*). A probe for human β-actin was used as a control. Longer exposures of the autoradiogram revealed that some of the tumor cells expressed *KAI1*, but in amounts much smaller than those expressed by the normal prostate tissue.

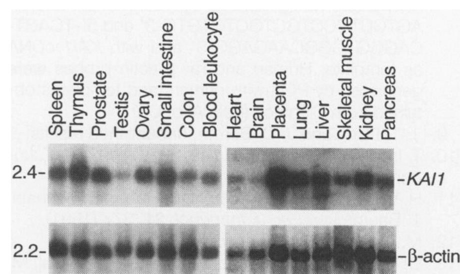

Fig. 3. Expression of *KAI1* in various human tissues. The multiple-tissue Northern blots were obtained from Clontech. The blots had 2 μg of polyadenylated RNA in each lane and were hybridized to the *KAI1* probe (*8*). A probe for human β-actin was used as a control.

involved in cell penetration and motility, which are in vitro parameters for metastasis (*23*). Likewise, reduction or loss of *ME491* expression is associated with increased metastatic ability of human malignant melanoma (*22*). Further studies are needed to clarify the functional roles of these genes in tumor metastasis.

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6. AT6.1 is a highly metastatic Dunning rat prostate cancer cell line. Microcell hybrid AT6.1-11-1 contains a fragment of human chromosome 11 from the centromere to region p13 and was suppressed for metastatic ability. Microcell hybrids AT6.1-11-2 and AT6.1-11-3 contain smaller fragments of human chromosome 11 from the centromere to region p11.2 and were not suppressed for metastatic ability (*4*).
7. Clones were obtained as follows: Polyadenylated RNA was isolated from exponentially growing AT6.1-11-1 cells with the FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). A cDNA library for AT6.1-11-1 was constructed in a pSPORT 1 vector with the SuperScript Plasmid System (Gibco BRL). Human Alu sequence primer Alu 559 (*5*) was used to amplify genomic DNA from the suppressed hybrid AT6.1-11-1 and from the nonsuppressed clone AT6.1-11-2 by PCR. The multiple Alu-PCR fragments from AT6.1-11-1 were cloned into a T-tailed vector, pCR1000 (Invitrogen). Individual clones corresponding to each fragment of these Alu-PCR products were isolated after comparison of the sizes of the Alu-PCR products to molecular weight markers in agarose gel stained with ethidium bromide. Eleven fragments unique to AT6.1-11-1 were labeled by random priming (Gibco BRL) and were used to screen 5×10^4 recombinants of the cDNA library under stringent wash conditions [65°C in 0.1× standard saline citrate (SSC) and 0.1% SDS for 30 min]. Five independent clones were obtained, and their inserts were sequenced with the Sequenase kit (U.S. Biochemical, Cleveland, OH). DNA sequences were analyzed with the GCG package, version 7.3 (Genetics Computer Group, Inc., Madison, WI).
8. Southern and Northern blots were hybridized to the *KAI1* probe at 68°C in QuikHyb hybridization solution (Stratagene), washed at 68°C for 30 min in 0.1× SSC and 0.1% SDS, and autoradiographed. The *KAI1* probe was generated by PCR with primers 5′-AGTCCTCCCTGCTGCTGTGTG-3′ and 5′-TCAGTCAGGGTGGGCAAGAGG-3′ and with *KAI1* cDNA as template. Human and rat β-actin probes were generated by PCR with primers and templates obtained from Clontech (Palo Alto, CA).
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14. *KAI1* cDNA was cloned into plasmid pCMVneo, in which transcription is driven by the constitutive human cytomegalovirus promoter (*13*). The resultant plasmid pCMV-KAI1 was transfected into AT6.1 cells by the calcium phosphate precipitate method. Individual transfectants were isolated in selection medium. Exponentially growing vector or *KAI1* transfectants were collected by scraping, and cell clumps were broken up by gentle pipetting. The cell suspension was placed in a tube and allowed to stand at room temperature for 30 min. Cells from the supernatant suspension were collected, washed, and resuspended in cold phosphate-buffered saline at 10^6 cells/ml. Male Ncr *nu/nu* nude mice 4 to 5 weeks old were injected with 10^5 cells (0.1 ml) subcutaneously at right and left midlateral sites, about one-fourth of the distance from the base of the skull to the base of the tail. About 6 weeks after injection, the tumors were weighed and the lungs were inflated with Bouin's solution. Tumor foci on the surfaces of lungs were scored under a dissecting microscope.
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29. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Long-Range Motional Restrictions in a Multidomain Zinc-Finger Protein from Anisotropic Tumbling

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Structural characterization of biomolecules in solution by nuclear magnetic resonance (NMR) spectroscopy is based primarily on the use of interproton distances derived from homonuclear cross-relaxation experiments. Information about short time-scale dynamics, on the other hand, is obtained from relaxation rates of heteronuclear spin pairs such as ^{15}N-^{1}H. By combining the two types of data and utilizing the dependence of heteronuclear NMR relaxation rates on anisotropic diffusional rotational tumbling, it is possible to obtain structural information about long-range motional correlations between protein domains. This approach was applied to characterize the relative orientations and mobilities of the first three zinc-finger domains of the *Xenopus* transcription factor TFIIIA in aqueous solution. The data indicate that the motions of the individual zinc-finger domains are highly correlated on time scales shorter than 10 nanoseconds and that the average conformation of the three-finger polypeptide is elongated.

Proteins of modular architecture, assembled from independently folded domains, are common in eukaryotes and play an important role in such fundamental events as transcriptional regulation, signal transduction, and blood coagulation. Characterization of the interdomain spatial interactions and motions is essential for understanding the function and biological activity of modular proteins. NMR spin relaxation measurements offer the potential for characterizing the flexibility and overall solution structure of multidomain proteins, even when most interdomain proton-proton distances exceed the 5 Å limit for observation of the nuclear Overhauser effect (NOE).

Orientational correlations between protein domains can induce anisotropic overall

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