

## SHORT COMMUNICATION

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## Molecular cloning and expression analysis of the human *DA41* gene and its mapping to chromosome 9q21.2–q21.3

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**Abstract** *DA41* was previously identified as one of the DAN-binding proteins, via a yeast-based two-hybrid screening strategy. In the present study, we cloned a human homolog of *DA41* cDNA. Structural analysis revealed that human *DA41* cDNA consisted of 2,861 nucleotides in length and encoded a protein of 589 amino acids, with a predicted molecular mass of 62.4kDa. Human *DA41* exhibited an 86% amino acid sequence identity to rat *DA41*, indicating the evolutionarily conserved structure and function of *DA41*. A database search for *DA41*-related protein(s) identified mouse *PLIC-1*, *PLIC-2*, frog *XDRP1*, and yeast *DSK2*. *DA41* and each *DA41*-related protein contain a ubiquitin-like domain in their amino-terminal regions. *DA41* was expressed ubiquitously in adult human tissues, with relatively higher levels in pituitary gland, adrenal gland, kidney, thymus, and placenta. Fluorescence in situ hybridization (FISH) revealed that *DA41* was mapped to human chromosome 9q21.2–q21.3, a position overlapping the candidate tumor suppressor locus for bladder cancer.

**Key words** Chromosome 9q · *DA41* · DAN · FISH · Ubiquitin-like protein

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The sequence data described in this article have been deposited with the EMBL/GenBank/DDBJ Data libraries under Accession no. AB035275

### Introduction

The *DAN* gene was originally cloned as one of the genes whose expression was significantly reduced in rat fibroblast 3Y1 cells transformed with Rous sarcoma virus (SR-3Y1) (Ozaki and Sakiyama 1993). The expression of *DAN* was also downregulated in rodent fibroblasts transformed with a variety of oncogenes (Ozaki and Sakiyama 1993; Ozaki et al. 1996). Overexpression of *DAN* in SR-3Y1 cells markedly suppressed the transformed phenotypes of the recipient cells, indicating the growth- and tumor-suppressive properties of *DAN* protein (Ozaki and Sakiyama 1994). Interestingly, *DAN* is a secreted glycoprotein (Nakamura et al. 1997; Stanley et al. 1998) and possesses a characteristic cysteine-knot structure common to the *DAN*/Cerberus family, which includes *Gremlin/Drm* (Hsu et al. 1998). Like *DAN*, the expression of *Drm* was significantly decreased in various transformed cells and its gene product showed a growth-suppressive activity in vitro (Topol et al. 1997). Recently, Hsu et al. (1998) demonstrated that the *DAN*/Cerberus family can interact with *BMP2* and block *BMP2* signalling in *Xenopus* early embryos. Similar results were also obtained in mammalian cell systems (Pearce et al. 1999).

*DA41* was initially identified as a new cellular protein which can associate with *DAN*, in a study that used a yeast two-hybrid screening of an adult rat lung cDNA library (Ozaki et al. 1997). The interaction between *DAN* and *DA41* was mediated through the amino-terminal domain and the cysteine-knot region of *DAN*. The expression of *DA41* was regulated in a cell cycle-dependent manner. In the present study, we performed cDNA cloning and characterization of human *DA41*, including its expression and its chromosomal mapping.

### Methods, results, and discussion

In order to isolate a full-length human homolog of *DA41* cDNA, we screened a human nigra cDNA library (approximately  $5 \times 10^6$  independent phage clones), using a radio-

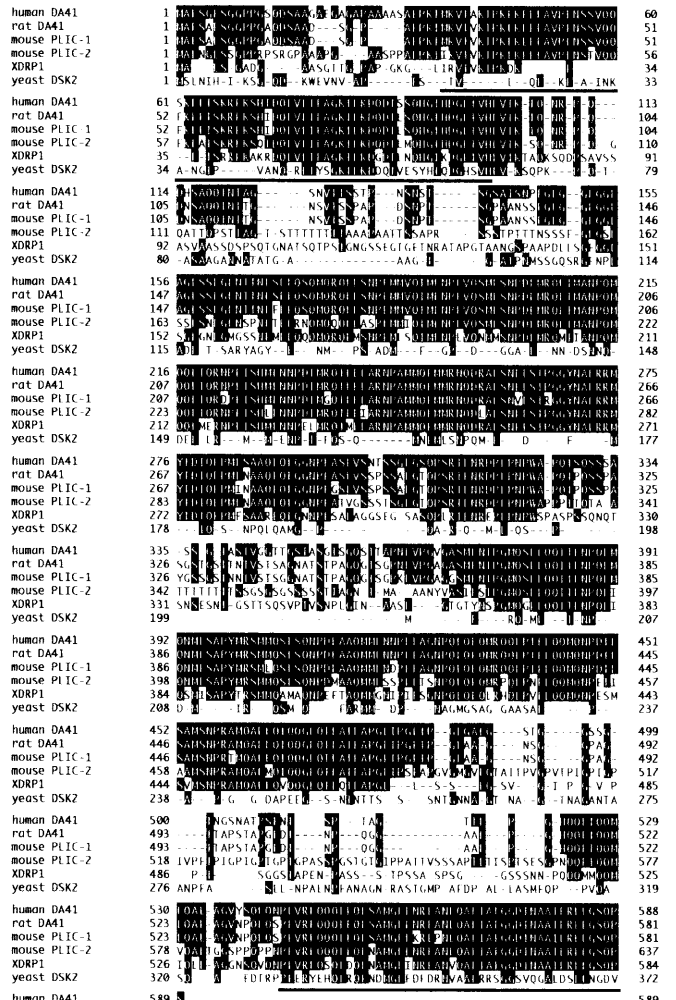
labeled full-length rat *DA41* cDNA as a probe. Among seven positives, we picked up two independent clones ( $\lambda$ 41-7 and  $\lambda$ 41-9) which harbored the longer cDNA inserts. Because the putative initiation codon was not located within the nucleotide sequences of these cDNA clones, the 5'-region of DA41-7 cDNA was radiolabeled and used to screen a human lung cDNA library (approximately  $5 \times 10^6$  recombinants). This screening yielded four independent positive signals. Sequence analysis revealed that the clone  $\lambda$ 41-11 contained a possible initiation codon (at position +1), which fulfilled Kozak's criteria for an initiation codon (Kozak 1987), and was preceded by an in-frame stop codon at position -30. The assembled human *DA41* cDNA consisted of 2,861 nucleotides and contained a single open reading frame of 1,767 nucleotides. The 3'-untranslated region (UTR) contained a consensus polyadenylation signal

139 GATTCGGTACGACACTCTTTGAGGAGTTGAGCTGGTGTAACTCAAGC 321  
 134 CCGATACGTCGAGTACTCTCTGCGAGCTGGGGCTGGCTTGTCTGTGCTGCTC 401  
 140 TCCCTCGCTCGTCCGCTGCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 41  
 1 AATCGGAGTGTAAAGTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 46  
 1 MAESGESGCGPPSPSSQQSAAAGA 78  
 64 GAAAGTCTGGTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 128  
 72 TEGAGAGAPAAAASAEPKREKVV 140  
 81 GGACAGCTCGAAGAAGAAAGGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 180  
 41 AATPTKCEKFAHWDFYVDF 198  
 181 TTTAGCAAAAATTTGAGGCTTTTAACTTCAAGCTGAGCTGCTGCTGCTGCTGCTG 240  
 81 SKREITGKPKFDF 260  
 241 GGTCGAAGATTTGAGAGTAAAGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 300  
 83 AKKLLIDKDDTSSQNSIMDL 320  
 381 AGCTGTCGCTTCTCAAAACAGAAAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 360  
 181 TVHIVLTKTRKQKRRPQGHSAQ 420  
 381 AATAGAGTGAAGCTGCTTCACTAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 470  
 123 NTAGSVAFFSPTNSNSTS 510  
 421 GTCCTCACTAGCAAGCTTTTGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 480  
 143 S ATSNPFGGGIGLGLASLS 500  
 481 TGGGCTGAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 540  
 161 I GINTYTPFSELGSGMQRQL 580  
 541 TGTAGCTGAAATGATGGTGAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 600  
 181 SNPEHMKWQIMENPPFYQSMES 700  
 601 AAATCGTCAGCTGATGAGATTAATGATGCTGCTGCTGCTGCTGCTGCTGCTGCTG 660  
 781 NPDLHKGQIMANPPHQQL 720  
 661 AAAATCTGAGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 720  
 221 AAATCTGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 740  
 721 ANCTCAGAACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 760  
 171 LARNPAMWQKRNKVP 780  
 781 ANCTAGAAAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 840  
 281 NLISLQDGNALSKWVP 860  
 841 TCAAGTATGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 900  
 281 EPMLESAAQQEGGKPPASLV 980  
 981 AGCAATAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1060  
 381 N TSSGSGGSQPTENRKP 1020  
 981 CCGATATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1080  
 321 PNPMPAQDSSSSASSGTA 1140  
 1021 ACTGTGGTCACTAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1080  
 341 TVGGITGSSASTAGCTGCTG 1100  
 1081 CCMAATTTGGTGGTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1140  
 351 PNPVPPQVGASMHNTPGMQL 1160  
 1141 TTCACAAATACCTGAAAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1200  
 381 LQDITLPNGQENHMASAPP 1240  
 1201 AGAGCATGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1260  
 481 RSMHMQSLSQNPDDLAARQHM 1280  
 1261 ANCTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1320  
 421 NPLPFAQKWPQLFQKMWPP 1340  
 1321 TTTCAGCAAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1380  
 441 F L Q K W P P T S A H N P P A H K 1400  
 1381 TAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1440  
 443 G A L L S Q I Q G L Q T A H H 1460  
 1441 AGTCCGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1500  
 483 P P R P D I G A L D S T G S S G T 1520  
 1501 AATGATTTAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1560  
 581 N S N A T P S E S P A G T T P 1620  
 1561 GSAATGCAAGTATTAGAGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1620  
 523 C H Q Q I Q M L O A I A G V Y S Q L 1640  
 1621 CAGATTAAGATCAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1680  
 543 Q N P E R F Q Q Q L E Q I S A M G P L 1660  
 1681 AACCTGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1740  
 563 N R E A N L D A I A I T C G D I N A I 1680  
 1741 GAAAGCTAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 1800  
 583 E R L E C S Q P S 1680  
 1801 TTTATTTCTAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1860  
 1861 TGGATTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1920  
 1861 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1980  
 1981 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2040  
 2041 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2100  
 2101 AAGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2160  
 2161 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2220  
 2221 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2280  
 2281 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2340  
 2341 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2400  
 2401 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2460  
 2461 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2520  
 2521 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2580  
 2581 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2640  
 2641 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2700  
 2701 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2760  
 2761 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2820  
 2821 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2880  
 2881 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2940  
 2941 TGTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 3000

**Fig. 1.** The nucleotide and deduced amino acid sequences of human *DA41* cDNA. The position of the stop codon is indicated by the asterisk and the putative polyadenylation signal is underlined. The nucleotide sequence reported here will appear in EMBL/GenBank/DBJ Data libraries under Accession no. AB035275

AATAAAA at position +2,111 and a poly(A) sequence at the end (Fig. 1). The *DA41* gene possibly exists as a single copy in the human genome, as examined by genomic Southern analysis (data not shown). Human *DA41* encoded a protein consisting of 589 amino acid residues with a predicted molecular mass of 62.4kDa. Human DA41 exhibited 86% identity to rat DA41 at the protein level (Fig. 2).

A recent database search for DA41-related protein(s) identified mouse PLIC-1 and PLIC-2 (Wu et al. 1999), frog XDRP1 (Funakoshi et al. 1999), and yeast DSK2 (Biggins et al. 1996). PLIC-1 and PLIC-2 are closely related in sequence (higher than 80% sequence identity between them). Human DA41 exhibited 81%, 61%, 57%, and 20% amino acid sequence identity to PLIC-1, PLIC-2, XDRP1, and DSK2, respectively (Fig. 2). PLIC-1 and PLIC-2 were identified to



**Fig. 2.** The amino acid sequence alignment of human DA41 with rat DA41 (accession no. D87950) and the other previously reported DA41-related proteins; mouse PLIC-1 (accession no. AF177345), mouse PLIC-2 (accession no. AF177346), frog XDRP1 (accession no. AB030502), and yeast DSK2 (accession no. L40587). Identical amino acid residues are printed in white type on black background. Gaps are introduced to maximize the alignment. N-terminal UBQ and C-terminal UBA regions are underlined

interact with the cytoplasmic tail of integrin-associated protein (IAP) and mediated the interaction between IAP and vimentin-containing intermediate filaments (Wu et al. 1999). XDRP1 was associated with the amino-terminal region of cyclin A and inhibited the degradation of cyclin A (Funakoshi et al. 1999). Overproduction of DSK2 induced a mitotic defect with short spindle (Biggins et al. 1996). DA41 and each DA41-related protein share evolutionarily conserved amino-terminal and carboxy-terminal regions which are homologous to ubiquitin (UBQ domain) and ubiquitin-associated region (UBA domain), respectively (Wu et al. 1999). Human DA41 was 94%, 88%, 58%, and 37% identical to the UBQ domains of PLIC-1, PLIC-2, XDRP1, and DSK2, respectively. Similarly, human DA41 exhibited 96%, 98%, 91%, and 38% sequence identity to the UBA domains of PLIC-1, PLIC-2, XDRP1, and DSK2, respectively.

We analyzed the expression of the human *DA41* gene in adult tissues. Human RNA master blot (Clontech, Palo Alto, CA, USA) was hybridized under the conditions rec-

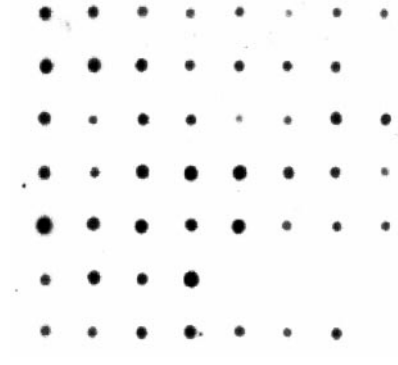
ommended by a manufacturer. A random primer-labeled 3'-untranslated region of human *DA41* cDNA was used as a probe in this experiment. As shown in Fig. 3a, human RNA master blot analysis revealed the ubiquitous expression of the *DA41* gene. Abundant expression was detected in adult pituitary gland, adrenal gland, kidney, thymus, and placenta. Ubiquitous expression of the *DA41* gene was also observed in adult rat tissues (Ozaki et al. 1997). Similarly, *PLIC-1* and *PLIC-2* were expressed in most mouse tissues and cell types (Wu et al. 1999).

In order to determine the chromosomal location of *DA41*, a human PAC library was screened with PCR, using primers specific for *DA41* (5'-ACTGCATGCATCACT-TCTGC-3') and (5'-TGGACAGATGCAGGACAAA-3'). A single PAC clone, which was confirmed to contain *DA41* by Southern blot hybridization, was obtained. This PAC clone was then labeled with biotin and used for direct R-banding fluorescence in situ hybridization (FISH) (Takahashi et al. 1990, 1991). As shown in Fig. 3b, twin

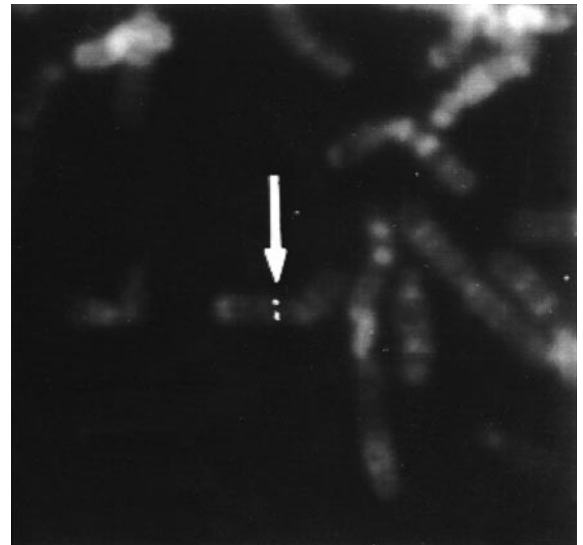
**Fig. 3. a** The tissue distribution of *DA41* was examined using human RNA master blot (Clontech). The filter was hybridized with a radio-labeled cDNA probe encoding the 3'-untranslated region of human *DA41* under the conditions recommended by the manufacturer. **b** Fluorescence in situ hybridization (FISH) mapping of the *DA41* gene to 9q21.2-q21.3. A PAC clone containing the *DA41* gene was used for the chromosomal localization of *DA41* by FISH. Metaphase spreads prepared from normal peripheral blood lymphocytes were hybridized with a biotin-labeled probe. *Arrow* indicates the positive signals on 9q21.2-q21.3

**a**

	1	2	3	4	5	6	7	8
<b>A</b>	whole brain	amygdala	caudate nucleus	cerebellum	cerebral cortex	frontal lobe	hippocampus	medulla oblongata
<b>B</b>	occipital lobe	putamen	substantia nigra	temporal lobe	thalamus	nucleus accumbens	spinal cord	
<b>C</b>	heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
<b>D</b>	testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
<b>E</b>	kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
<b>F</b>	appendix	lung	trachea	placenta				
<b>G</b>	fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	



**b**



signals were observed on the q21.2–q21.3 band of chromosome 9.

Funakoshi et al. (1999) have found that frog XDRP1, which exhibits extensive homology with DA41, can associate with cyclin A-CDK complex. Overproduction of XDRP1 inhibited the degradation of cyclin A and blocked cell division. These observations strongly suggest that DA41 and XDRP1 could share a similar function in cell cycle regulation. It is intriguing to note that the expression of *DA41* was regulated in a cell cycle-dependent manner in synchronized 3Y1 rat fibroblasts (Ozaki et al. 1997). Recently, two candidate tumor suppressor loci for bladder cancer were identified on chromosome 9; 9p21–q21 and distal chromosome 9q (Simoneau et al. 1996). In addition, the proximal region of chromosome 9q was deleted in bladder cancer (Bernues et al. 1993). Because *DA41* was mapped within or near the candidate locus for bladder cancer, *DA41* may be involved in bladder carcinogenesis. To test this possibility, information on structural alteration(s) within *DA41* in primary bladder cancers is necessary.

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