Identifying the role of Wilms tumor 1 associated protein in cancer prediction using integrative genomic analyses

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Abstract. The Wilms tumor suppressor, WT1 was first identified due to its essential role in the normal development of the human genitourinary system. Wilms tumor 1 associated protein (WTAP) was subsequently revealed to interact with WT1 using yeast two-hybrid screening. The present study identified 44 complete WTAP genes in the genomes of vertebrates, including fish, amphibians, birds and mammals. The vertebrate WTAP proteins clustered into the primate, rodent and teleost lineages using phylogenetic tree analysis. From 1,347 available SNPs in the human WTAP gene, 19 were identified to cause missense mutations. WTAP was expressed in bladder, blood, brain, breast, colorectal, esophagus, eye, head and neck, lung, ovarian, prostate, skin and soft tissue cancers. A total of 17 out of 328 microarrays demonstrated an association between WTAP gene expression and cancer prognosis. However, the association between WTAP gene expression and prognosis varied in distinct types of cancer, and even in identical types of cancer from separate microarray databases. By searching the Catalogue of Somatic Mutations in Cancer database, 65 somatic mutations were identified in the human WTAP gene from the cancer tissue samples. These results suggest that the function of WTAP in tumor formation may be multidimensional. Furthermore, signal transducer and activator of transcription 1, forkhead box protein O1, interferon regulatory factor 1, glucocorticoid receptor and peroxisome proliferator-activated receptor γ transcription factor binding sites were identified in the upstream (promoter) region of the human WTAP gene, suggesting that these transcription factors may be involved in WTAP functions in tumor formation.

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Introduction

The Wilms tumor suppressor gene WT1 was first identified due to its essential role in the normal development of the human genitourinary system (1). WT1 functions as a transcription factor regulating target gene expression (1). In addition, WT1 was revealed to regulate the expression of genes involved in the Wnt signaling pathway via a genome-wide screening analysis (2).

Wilms tumor 1 associated protein (WTAP) was demonstrated to interact with WT1 using a yeast two-hybrid screening (3). WTAP and WT1 were observed to localize to the nucleoplasm and nuclear speckles, where they were partially co-localized with splicing factors (3). WTAP is the mammalian homolog of the Drosophila gene female-lethal(2) D [fl(2)D], and fl(2)D is involved in activating female-specific patterns of alternative splicing of sex-lethal and transformer pre-mRNA (4,5). Previous studies demonstrated that WTAP-null and heterozygous mice succumbed between embryonic day 6.5 and 10.5, and exhibited marked defects in cell proliferation, which resulted in defects in endoderm and mesoderm formation (6,7). Furthermore, it has been demonstrated in mice that WTAP is required for G_2/M cell cycle transition by stabilizing the cyclin A2 mRNA and, thus, is vital in early development (6). In addition, WTAP is involved in the function of human spliceosomes (8). Recently, it was demonstrated that WTAP and virilizer are subunits of the N6-methyladenosine methylation complex, which regulates mRNA stability (9,10).

A limited number of studies have been performed on the role of WTAP in tumor genesis. WTAP is overexpressed in cholangiocarcinoma and WTAP expression was observed to correlate with metastasis (11). In addition, WTAP was demonstrated to be overexpressed in glioblastoma, and regulated glioblastoma cell migration and invasion (12). Bansal *et al* (13) identified WTAP as an oncogenic protein in acute myeloid

leukemia. Carbonic anhydrase 4, a novel tumor suppressor in colorectal cancer, inhibited the Wnt signaling pathway by targeting the WTAP-WT1-transducin β-like 1 axis (14). The aim of the present study was to investigate the role of WTAP in tumor formation by identifying novel WTAP genes in vertebrate genomes. The expression of these genes in healthy and tumor tissue samples was determined, and functionally relevant single nucleotide polymorphisms (SNPs) and somatic mutations in WTAP were identified. Conserved transcription factor binding sites within the promoter region of the human WTAP gene were identified. Furthermore, meta-analysis of the prognostic value of WTAP gene expression in various cancers was performed.

Materials and methods

Identification of novel WTAP genes in vertebrate genomes and transcription factor-binding sites. The DNA and amino acid sequences of novel vertebrate WTAPs were obtained by searching Ensembl genome databases (ensembl.org/index.html) using orthologous and paralogous associations. The prospective WTAP sequences were confirmed using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) (15-18). Conserved transcription factor-binding sites in the promoter regions of the human WTAP gene were identified from the SABiosciences' proprietary database, DECODE (Qiagen, Inc., Valencia, CA, USA), which combines text mining with the University of California, Santa Cruz genome browser data (19-21).

Comparative proteomic analyses of WTAP proteins. The amino acid sequences of identified vertebrate WTAPs were aligned using ClustalW (ebi.ac.uk/Tools/msa/). A maximum likelihood (ML) tree of vertebrate WTAPs was constructed using Molecular Evolutionary Genetics Analysis version 5.05 (megasoftware.net/) with the optimal model (Kimura 2-parameter model). The relative support of internal nodes was determined by bootstrap analyses with 1,000 replications for ML reconstructions (22). The program, codeml within the Phylogenetic Analysis by ML version 4.7 software package (abacus.gene.ucl.ac.uk/software/paml.html) was used to investigate whether WTAP proteins were positively selected (23).

Identification of functionally relevant SNPs in the human WTAP gene and somatic mutations in human cancer. The functionally relevant SNPs of the human WTAP gene were extracted from the Ensembl genome databases and the Short Genetic Variations database (ncbi.nlm.nih.gov/snp), as previously described (15-21). The SNPs causing missense mutations were then identified. The somatic mutations of the human WTAP gene in cancer tissues were extracted from the Catalogue of Somatic Mutations in Cancer (COSMIC) database (cancer.sanger.ac.uk/cosmic), which mines somatic mutations in complete cancer genomes (24).

In silico expression analyses of the human WTAP gene. Expression profiles of the human WTAP gene in normal tissues were obtained from the GeneAnnot (genecards.weizmann.ac.il/geneannot/index.shtml) (25) and ArrayExpress (ebi.ac.uk/arrayexpress/) databases (26).

Meta-analysis of the prognostic value of human WTAP gene expression in cancer tissues. The expression of the WTAP gene and the biological association between gene expression and prognosis were determined by inputting human WTAP gene (NP_001257460) into the PrognoScan database (prognoscan.org/) (27).

Results

Comparative proteomics of WTAP proteins identified in vertebrate genomes. WTAP DNA and protein sequences were collected from the Ensembl genome database and confirmed by BLASTing. Completed WTAP genes were identified in the following genomes: Human, chimpanzee, gibbon, macaque, gorilla, orangutan, olive baboon, vervet monkey, marmoset, tarsier, bush baby, armadillo, sloth, squirrel, elephant, guinea pig, mouse, rat, pika, horse, microbat, ferret, dolphin, dog, pig, sheep, cow, alpaca, chicken, duck, turkey, flycatcher, zebra finch, Chinese softshell turtle, anole lizard, spotted gar, Amazon molly, platyfish, stickleback, tilapia, medaka, cave fish and zebrafish. In the armadillo genome, two WTAP genes were identified. The maximum likelihood method was used to construct the phylogenetic tree of vertebrate WTAPs (Fig. 1). The vertebrate WTAP genes clustered into the primate, rodent and teleost lineages. Furthermore, site-specific analysis for positive selection with six models of codon substitution, M0 (one-ratio), M1a (nearly neutral), M2a (positive selection), M3 (discrete), M7 (β), and M8 (β and ω) were performed in vertebrate, mammalian, bird, reptile and teleost lineages. No sites were identified under positive selection with any models in the various WTAP groups. Therefore, it was concluded that WTAP proteins were under purifying selection (data not shown).

Expression profile of the human WTAP gene. Investigation of available microarray data revealed that the human WTAP gene was predominantly expressed in the following tissues: Bone marrow, whole blood, lymph node, brain, cerebellum, retina, spinal cord, heart, smooth muscle, skeletal muscle, small intestine, colon, adipocyte, kidney, liver, lung, pancreas, thyroid, salivary gland, adrenal gland, skin, ovary, uterus, placenta, prostate and testis. In addition, the human WTAP gene was expressed in the following types of cancer: Bladder, blood, brain, breast, colorectal, esophagus, eye, head and neck, lung, ovarian, prostate, skin and soft tissue.

Comparative genomics on the human WTAP gene. Signal transducer and activator of transcription 1 (STAT1), forkhead box protein O1 (FOXO1), interferon regulatory factor 1 (IRF1), glucocorticoid receptor and peroxisome proliferator-activated receptor γ (PPARγ) transcription factor binding sites were identified in the upstream (promoter) region of the WTAP gene.

Functionally relevant SNP identification in the human WTAP gene. A total of 1,347 available SNPs were identified in the human WTAP gene. Among these, 19 SNPs were functionally relevant, causing missense mutations (Table I).

Identification of somatic mutations of the WTAP gene in human cancer. By searching the COSMIC database,

Table I. Functionally relevant SNP identification in the human WTAP gene.

SNP	Chr 6 position sequence	Sequence	Type	Amino acid
rs1543500	159,755,455(+)	GGCAG(G/T)GAAAA	Missense	S (Ser) ⇒ R (Arg)
rs112093927	159,753,489(+)	GAAGT(A/G)TCGAA	Missense	C (Cys) ⇒ Y (Tyr)
rs140439442	159,755,519(+)	AGAAA(A/G)CAGTG	Missense	A (Ala) ⇒ T (Thr)
rs146208471	159,755,499(+)	CAGTC(A/G)TGACC	Missense	H (His) ⇒ R (Arg)
rs144625269	159,755,303(+)	ACAGG(A/G)AGGGC	Missense	E (Glu) ⇒ K (Lys)
rs148080007	159,739,015(+)	CTTCA(A/G)AGTTA	Missense	K (Lys) ⇒ R (Arg)
rs149103382	159,755,447(+)	CTCCC(A/G)CGGGC	Missense	T (Thr) ⇒ A (Ala)
rs150215853	159,743,764(+)	AGCAA(C/G)CAAGG	Missense	T (Thr) ⇒ S (Ser)
rs187127278	159,755,555(+)	GTTCC(C/T)GCCAC	Missense	R (Arg) ⇒ C (Cys)
rs373161821	159,755,405(+)	GTTAC(A/G)TAAAT	Missense	V (Val) ⇒ I (Ile)
rs375163417	159,755,312(+)	GCAAC(A/G)CAACC	Missense	T (Thr) ⇒ A (Ala)
rs375840138	159,755,421(+)	CAGTG(C/T)GGGGT	Missense	A (Ala) ⇒ V (Val)
rs528166112	159,755,353(+)	GGTAA(A/T)AAGTC	Missense	N (Asn) ⇒ K (Lys)
rs532857576	159,748,230(+)	CGAGC(A/G)TTGCC	Missense	V (Val) ⇒ I (Ile)
rs543667028	159,755,190(+)	AGCTT(C/T)TGAAC	Missense	S (Ser) ⇒ F (Phe)
rs546830649	159,755,349(+)	GAATG(A/G)TAATA	Missense	G (Gly) ⇒ D (Asp)
rs558627827	159,742,104(+)	ATGAA(A/G)CATAT	Missense	A (Ala) ⇒ T (Thr)
rs560214098	159,748,326(+)	GCATC(A/G)TCTGC	Missense	Q (Gln) ⇒ K (Lys)
rs563264867	159,755,400(+)	TAGTG(A/G)TTACG	Missense	G (Gly) ⇒ D (Asp)

SNP, single nucleotide polymorphism; WTAP, Wilms tumor 1 associated protein; Chr, chromosome.

65 somatic mutations of the human WTAP gene were identified in various types of cancer tissue (Table II).

Meta-analysis of the prognostic value of human WTAP gene expression in cancer tissues. A total of 17 out of 328 microarrays identified an association between WTAP gene expressions and cancer prognosis (bladder cancers, 1/7; blood cancers, 0/37; brain cancers, 1/23; breast cancers, 5/110; colorectal cancers, 3/48; esophagus cancers, 0/1; eye cancers, 2/5; head and neck cancers, 0/6; lung cancers, 4/56; ovarian cancers, 0/25; prostate cancers, 0/1; skin cancers, 0/6; and soft tissue cancers, 1/3), $P<0.05$ (Table III) (28-38). In bladder, brain, eye and soft tissue cancers, reduced expression of the WTAP gene was associated with poor survival. However, an increased expression of the WTAP gene was associated with poor survival in lung cancer. Of the six breast cancer microarrays, reduced expression of the WTAP gene was associated with poor survival in two cases from the same database (GSE2990) and in the database GSE1456-GPL96, while increased expression of the WTAP gene was associated with poor survival in the GSE1456-GPL96 and GSE1456-GPL97 databases. Of the colorectal cancer microarrays, reduced expression of the WTAP gene was associated with poor survival in two cases (GSE17537 and GSE17538), while increased expression of the WTAP gene was associated with poor survival in the GSE14333 database.

Discussion

WT1 was first identified due to its essential role in the normal development of the human genitourinary system (1) and WTAP was identified as a protein that interacted with WT1 (3). A total of 44 complete WTAP genes were identified in the human, chimpanzee, gibbon, macaque, gorilla, orangutan, olive baboon, vervet monkey, marmoset, tarsier, bush baby, armadillo, sloth, squirrel, elephant, guinea pig, mouse, rat, pika, horse, microbat, ferret, dolphin, dog, pig, sheep, cow, alpaca, chicken, duck, turkey, flycatcher, zebra finch, Chinese softshell turtle, anole lizard, spotted gar, Amazon molly, platyfish, stickleback, tilapia, medaka, cave fish and zebrafish genomes. It was observed that WTAP genes were widely expressed in vertebrates, existing in fish, amphibians, birds and mammals. The phylogenetic tree revealed that the vertebrate WTAP proteins were clustered into the primate, rodent and teleost lineages. All vertebrate WTAPs are conserved according to the analysis of alignment and phylogenetic tree construction. Furthermore, the vertebrate WTAPs were under purifying selection. These results suggest that WTAP performs an essential physiological role in all vertebrates.

WTAP was predominantly expressed in bone marrow, whole blood, lymph node, brain, cerebellum, retina, spinal cord, heart, smooth muscle, skeletal muscle, small intestine, colon, adipocyte, kidney, liver, lung, pancreas, thyroid, salivary gland, adrenal gland, skin, ovary, uterus, placenta, prostate and testis. The expression pattern of WTAP appeared to be ubiquitous, which is indicative of a housekeeping role. By contrast, WT1 is expressed at low levels in only the spleen, heart, gonad and kidney (3). A total of 19 SNPs that cause missense mutations were identified in the human WTAP gene; however, it remains unclear whether these SNPs affect the physiological or pathological functions of WTAP.

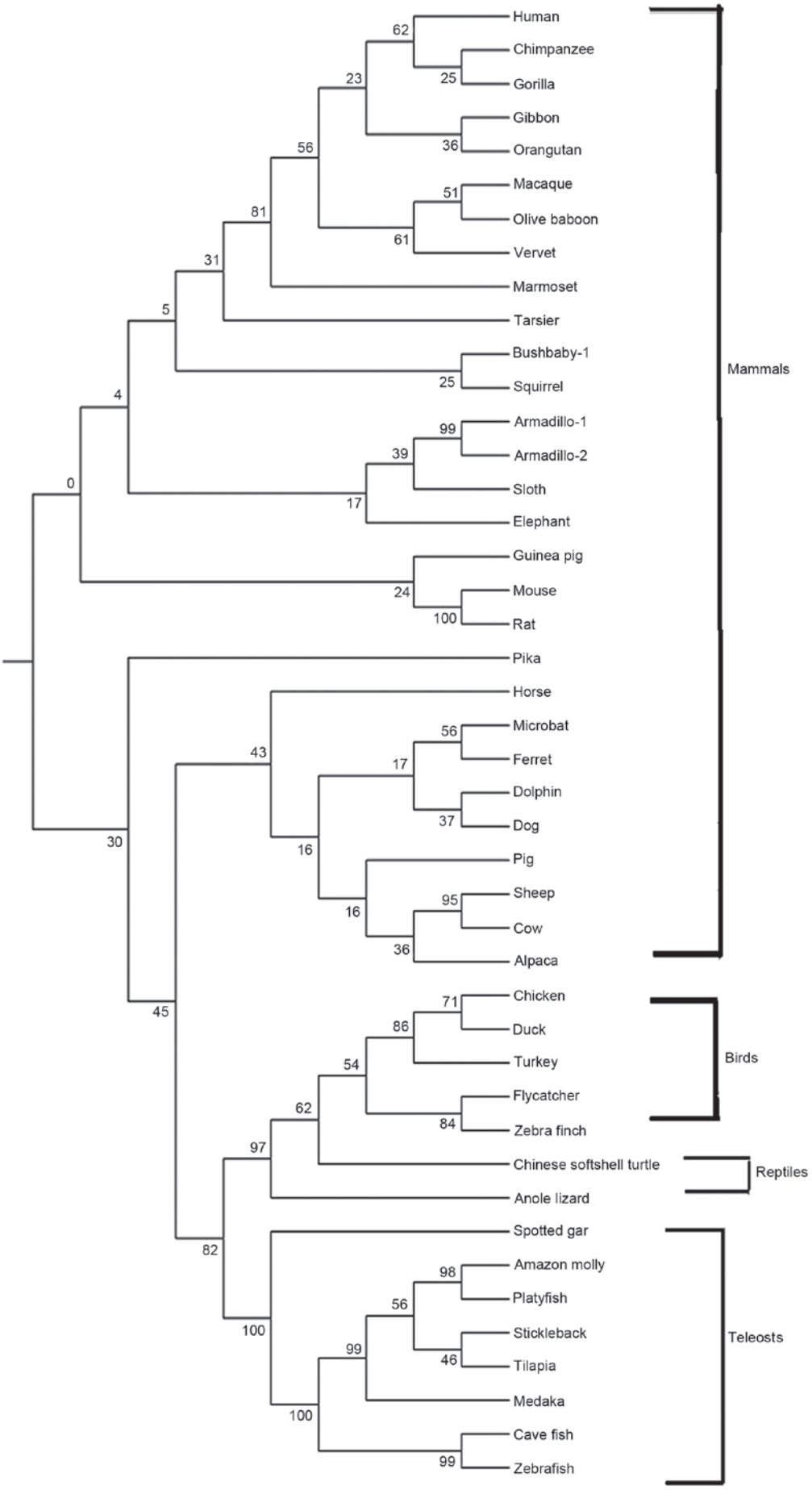

Figure 1. Phylogenetic tree of vertebrate WTAP. The phylogenetic tree of vertebrate WTAP was constructed using the maximum likelihood method. Vertebrate WTAP clustered into primate, rodent and teleost lineages.

Table II. Somatic mutations of WTAP in cancer tissues.

Position (AA)	Mutation (CDS)	Mutation (amino acid)	Mutation ID (COSM)	Count	Mutation type
12	c.34C>T	p.R12[a]	COSM207378	3	Substitution-nonsense
30	c.90G>A	p.W30[a]	COSM223136	1	Substitution-nonsense
32	c.96A>T	p.Q32H	COSM3622455	1	Substitution-missense
33	c.98A>G	p.Y33C	COSM4930374	1	Substitution-missense
35	c.104C>T	p.A35V	COSM4445039	1	Substitution-missense
44	c.132C>A	p.Y44[a]	COSM450822	1	Substitution-nonsense
51	c.152A>C	p.D51A	COSM450823	1	Substitution-missense
54	c.161G>A	p.G54D	COSM3860103	1	Substitution-missense
66	c.198G>A	p.Q66Q	COSM1075534	1	Substitution-coding silent
71	c.211C>T	p.R71C	COSM3860104	1	Substitution-missense
79	c.235C>T	p.R79[a]	COSM1441924	1	Substitution-nonsense
79	c.236G>A	p.R79Q	COSM450824	1	Substitution-missense
86	c.256G>A	p.E86K	COSM741338	1	Substitution-missense
91	c.271A>G	p.T91A	COSM1496256	1	Substitution-missense
95	c.283C>T	p.Q95[a]	COSM1621161	2	Substitution-nonsense
103	c.307C>T	p.P103S	COSM1075535	1	Substitution-missense
104	c.312C>T	p.S104S	COSM1441925	1	Substitution-coding silent
108	c.324G>C	p.L108L	COSM1311778	1	Substitution-coding silent
116	c.347C>T	p.A116V	COSM1311779	1	Substitution-missense
117	c.350T>A	p.I117N	COSM595210	1	Substitution-missense
119	c.355T>G	p.L119V	COSM1075536	1	Substitution-missense
135	c.405G>T	p.L135L	COSM450825	1	Substitution-coding silent
141	c.421G>A	p.E141K	COSM4806762	1	Substitution-missense
147	c.440T>C	p.F147S	COSM1075537	1	Substitution-missense
148	c.443C>T	p.T148M	COSM1232873	1	Substitution-missense
150	c.448G>A	p.D150N	COSM3023284	1	Substitution-missense
150	c.448G>C	p.D150H	COSM4828326	1	Substitution-missense
154	c.461G>A	p.G154E	COSM341987	2	Substitution-missense
156	c.466A>G	p.K156E	COSM5003572	1	Substitution-missense
156	c.467A>G	p.K156R	COSM420994	1	Substitution-missense
157	c.470T>C	p.L157S	COSM1545449	1	Substitution-missense
159	c.476C>A	p.A159E	COSM248361	1	Substitution-missense
159	c.476C>T	p.A159V	COSM3023287	1	Substitution-missense
162	c.485G>A	p.R162Q	COSM1441926	1	Substitution-missense
162	c.485G>C	p.R162P	COSM1487425	1	Substitution-missense
163	c.489G>A	p.M163I	COSM483632	1	Substitution-missense
163	c.489_494delGCTTAT	p.M163_L164delML	COSM242271	1	Deletion-in frame
164	c.491T>C	p.L164P	COSM1311780	1	Substitution-missense
209	c.627C>A	p.I209I	COSM3366370	1	Substitution-coding silent
211	c.632T>G	p.L211R	COSM4880307	1	Substitution-missense
212	c.635A>C	p.D212A	COSM595209	1	Substitution-missense
226	c.676C>T	p.Q226[a]	COSM4741494	1	Substitution-nonsense
231	c.693G>A	p.E231E	COSM207380	1	Substitution-coding silent
248	c.744C>T	p.A248A	COSM1270542	1	Substitution-coding silent
250	c.749G>C	p.S250T	COSM395321	1	Substitution-missense
255	c.763A>G	p.T255A	COSM4645558	1	Substitution-missense
255	c.765A>T	p.T255T	COSM741337	1	Substitution-coding silent
271	c.811A>T	p.S271C	COSM3023294	2	Substitution-missense
305	c.913T>C	p.S305P	COSM3622456	1	Substitution-missense
308	c.922G>T	p.G308W	COSM1441927	1	Substitution-missense
309	c.927T>G	p.N309K	COSM4160342	1	Substitution-missense

Table II. Continued.

Position (AA)	Mutation (CDS)	Mutation (Amino acid)	Mutation ID (COSM)	Count	Mutation type
314	c.941C>G	p.S314C	COSM1645128	1	Substitution-missense
334	c.1001C>T	p.A334V	COSM3928204	2	Substitution-missense
335	c.1003G>T	p.G335W	COSM595208	1	Substitution-missense
342	c.1020_1021delCT	p.P342fs[a]4	COSM3732402	1	Deletion-frameshift
343	c.1029G>A	p.T343T	COSM4741495	1	Substitution-coding silent
353	c.1058C>G	p.S353[a]	COSM1545448	1	Substitution-nonsense
367	c.1099G>T	p.A367S	COSM1075538	1	Substitution-missense
368	c.1104G>C	p.V368V	COSM4832802	1	Substitution-coding silent
374	c.1120C>T	p.R374[a]	COSM4741496	1	Substitution-nonsense
374	c.1121G>A	p.R374Q	COSM1075539	1	Substitution-missense
377	c.1130G>C	p.G377A	COSM4980694	1	Substitution-missense
384	c.1150G>A	p.G384S	COSM4637994	1	Substitution-missense
391	c.1173A>G	p.V391V	COSM3023307	1	Substitution-coding silent
393	c.1178G>T	p.G393V	COSM595207	1	Substitution-missense

Mutation (amino acid) refers to the change in amino acid sequence resulting from the mutation. [a]Stop codon. Count refers to the mutation number identified in the database. WTAP, wilms tumor 1 associated protein; AA, amino acid; CDS, coding DNA sequence; COSM, catalogue of somatic mutations.

WTAP and WT1 partially co-localize with splicing factors, and are distributed together in the nucleoplasm and in nuclear speckles (3). It has been demonstrated in numerous tumors that WT1 is a tumor suppressor, exerting effects including inhibiting cell proliferation and enhancing apoptosis (39-42). However, WTAP is an oncogene, which is overexpressed in cholangiocarcinoma (11), glioblastoma (12) and acute myeloid leukemia (13). In the present study, it was demonstrated that WTAP was expressed in bladder, blood, brain, breast, colorectal, esophagus, eye, head and neck, lung, ovarian, prostate, skin and soft tissue cancers. Of a total of 328 microarrays, 17 revealed an association between microarray WTAP expression and cancer prognosis (bladder cancers, 1; brain cancers, 1; breast cancers, 6; colorectal cancers, 3; eye cancers, 2; lung cancers, 4; and soft tissue cancers, 1). The majority of microarrays did not reveal an association between microarray WTAP expression and cancer prognosis. This may be due to a lack of WTAP expression information in the database. Notably, WTAP was not involved in all tumor types. In addition, it is notable that the association between WTAP expression and prognosis varied between the different cancer types, and even in identical cancers from separate databases. This suggests that the function of WTAP in these tumors may not be solely as an oncogene, but may be multidimensional (11-13). The differing WTAP expression in various tumors may be due to the distinct oncogenes or tumor suppressors stabilized by WTAP in particular tumors (9,10).

Furthermore, 65 somatic mutations of WTAP were identified in cancer tissues. The effects of these mutations on tumor formation remain to be elucidated and require future investigation. The results of the present study suggest that WTAP has a comprehensive and complex role in tumor formation. STAT1, FOXO1, IRF1, glucocorticoid receptor and PPARγ transcription factor binding sites were identified in the upstream (promoter) region of the WTAP gene. STAT1 is a cytoplasmic protein, which functions as a signal messenger and transcription factor in cellular responses to cytokines and growth factors (43). It exhibits anti-tumor functions via control of the immune system and promotion of tumor immune surveillance (44-46). FOXO1 is an important transcriptional regulator of cell proliferation and is considered to be essential for tumor growth and progression (47). Deregulation of FOXO1 promotes cell proliferation and tumorigenesis, and has thus become a primary target of tumorigenesis prevention (48,49). IRF1 is involved in the regulation of interferon α and β transcription, and it has been demonstrated that IRF1 gene deletion or rearrangement correlates with the development of human cancers (50,51). The glucocorticoid receptor is a member of the nuclear receptor family, which acts as a ligand-dependent transcription factor to regulate gene expression. In addition, the estrogen and androgen receptors are members of the nuclear receptor family. In breast cancer, the estrogen receptor drives cell growth, proliferation and metastasis, and the androgen receptor has a similar role in prostate cancer (52,53). These tumor-associated transcriptional factors may affect the expression of WTAP and contribute to tumor formation (12-14).

In conclusion, 44 complete WTAP genes were identified in vertebrate genomes. The vertebrate WTAP proteins clustered into the primate, rodent and teleost lineages. The association between WTAP gene expression and prognosis varied in distinct cancers, and even in identical cancers from separate microarray databases. Furthermore, a total of 65 somatic mutations were identified in the human WTAP gene from cancer tissue samples. The results of the present study suggest that the function of WTAP in tumor formation may be multidimensional.

Table III. Associations between microarray WTAP expression and cancer prognosis.

Author, year	Database	Cancer type	Subtype	Patient number	Endpoint	Cutpoint	P-value	Prognosis	Ref.
Kim, 2010	GSE5287	Bladder	Transitional cell carcinoma	165	Disease specific survival	0.11	0.025526	1	28
Freije, 2004	GSE4412-GPL97	Brain	Glioma	74	Overall survival	0.14	0.000331	1	29
Pawitan, 2005	GSE1456-GPL96	Breast		159	Overall survival	0.43	0.003810	1	30
Pawitan, 2005	GSE1456-GPL96	Breast		159	Relapse free survival	0.78	0.023849	2	30
Pawitan, 2005	GSE1456-GPL97	Breast		159	Relapse free survival	0.86	0.047352	2	30
Sotiriou, 2006	GSE2990	Breast		54	Distant metastasis free survival	0.15	0.039634	1	31
Sotiriou, 2006	GSE2990	Breast		62	Relapse free survival	0.13	0.038288	1	31
Jorissen, 2009	GSE14333	Colorectal		226	Disease free survival	0.85	0.011858	2	32
Smith, 2010	GSE17537	Colorectal		49	Disease specific survival	0.24	0.010751	1	33
Smith, 2010	GSE17538	Colorectal		55	Overall survival	0.25	0.010567	1	33
Laurent, 2011	GSE22138	Eye	Uveal melanoma	63	Distant metastasis free survival	0.49	0.049578	1	34
Laurent, 2011	GSE22138	Eye	Uveal melanoma	63	Distant metastasis free survival	0.21	0.007475	1	34
Tomida, 2009	GSE13213	Lung	Adenocarcinoma	117	Overall survival	0.89	0.000567	2	35
Tomida, 2009	GSE13213	Lung	Adenocarcinoma	117	Overall survival	0.90	0.012852	2	35
Okayama, 2012	GSE31210	Lung	Adenocarcinoma	204	Overall survival	0.88	0.043703	2	36
Bild, 2006	GSE3141	Lung	NSCLC	111	Overall survival	0.89	0.002284	2	37
Gobble, 2011	GSE30929	Soft tissue	Liposarcoma	140	Distant recurrence free survival	0.21	0.008282	1	38

The PrognoScan database was used to determine associations between expression of the WTAP gene and prognosis. $P<0.05$ was considered to indicate a statistically significant difference. WTAP, Wilms tumor 1 associated protein; NSCLC, non-small cell lung cancer.

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