# Integrative genomic analyses of the RNA-binding protein, RNPC1, and its potential role in cancer prediction

ZHIMING DING[1*], HAI-WEI YANG[2*], TIAN-SONG XIA[3*], BO WANG[4] and QIANG DING[3]

[1]Department of Neurosurgery, The Eastern Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong 510700; Departments of [2]Urology and [3]Breast Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029; [4]Department of Medical Oncology, The Eastern Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong 510700, P.R. China

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**Abstract.** The RNA binding motif protein 38 (RBM38, also known as RNPC1) plays a pivotal role in regulating a wide range of biological processes, from cell proliferation and cell cycle arrest to cell myogenic differentiation. It was originally recognized as an oncogene, and was frequently found to be amplified in prostate, ovarian and colorectal cancer, chronic lymphocytic leukemia, colon carcinoma, esophageal cancer, dog lymphomas and breast cancer. In the present study, the complete RNPC1 gene was identified in a number of vertebrate genomes, suggesting that RNPC1 exists in all types of vertebrates, including fish, amphibians, birds and mammals. In the different genomes, the gene had a similar 4 exon/3 intron organization, and all the genetic loci were syntenically conserved. The phylogenetic tree demonstrated that the RNPC1 gene from the mammalian, bird, reptile and teleost lineage formed a species-specific cluster. A total of 34 functionally relevant single nucleotide polymorphisms (SNPs), including 14 SNPs causing missense mutations, 8 exonic splicing enhancer SNPs and 12 SNPs causing nonsense mutations, were identified in the human RNPC1 gene. RNPC1 was found to be expressed in bladder, blood, brain, breast, colorectal, eye, head and neck, lung, ovarian, skin and soft tissue cancer. In 14 of the 94 tests, an association between RNPC1 gene expression and cancer prognosis was observed. We found that the association between the expression of RNPC1 and prognosis varied in different types of cancer, and even in the same type of cancer from the different databases used. This suggests that the function of RNPC1 in these tumors may be multidimensional. The sex determining region Y (SRY)-box 5 (Sox5), runt-related transcription factor 3 (RUNX3), CCAAT displacement protein 1 (CUTL1), v-rel avian reticuloendotheliosis viral oncogene homolog (Rel)A, peroxisome proliferator-activated receptor γ isoform 2 (PPARγ2) and activating transcription factor 6 (ATF6) regulatory transcription factor binding sites were identified in the upstream (promoter) region of the RNPC1 gene, and may thus be involved in the effects of RNPC1 in tumors.

*Correspondence to:* Dr Zhiming Ding, Department of Neurosurgery, The Eastern Hospital of the First Affiliated Hospital, Sun Yat-sen University, 183 Huangpu East Road, Guangzhou, Guangdong 510700, P.R. China
E-mail: dzming930@sohu.com

Dr Qiang Ding, Department of Breast Surgery, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing, Jiangsu 210029, P.R. China
E-mail: dingqiang@njmu.edu.cn

*Contributed equally

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## Introduction

RNA-binding proteins (RBPs) are known to play a crucial role in post-transcriptional regulation in gene expression, and regulate all aspects of RNA metabolism and function, such as polyadenylation, RNA splicing, transport, stability and translation; thus, they represent critical mechanisms for gene regulation in mammalian cells (1,2). They contain one or more RNA-binding motifs, such as the RNA recognition motif (RRM), the human heterogeneous nuclear ribonucleoprotein (hnRNP) K homology motif, the RGG box and the double-stranded RNA binding domain (dsRBD) motif. RRM is the most prevalent type of eukaryotic RNA-binding motif (3), which is composed of two submotifs, RNP1 and RNP2 (3). RBPs are involved in the expression of various genes responsible for regulating biological processes and cellular functions, and thus expected mutations or the aberrant production of RBPs can cause cancer progression (4,5).

The RNA binding motif protein 38 (RBM38, also known as RNPC1) gene is located on chromosome 20q13 and is expressed in a variety of tissues. It belongs to the RRM family of RBPs, is expressed as RNPC1a with 239 amino acids and as RNPC1b with 121 amino acids (6). RNPC1 plays pivotal roles in regulating a wide range of biological processes, ranging from cell proliferation and cell cycle arrest to cell myogenic differentiation (7,8). It is capable of regulating these biological processes by binding and stabilizing the mRNA of p21, p73,

Hu antigen R (HuR) and macrophage inhibitory cytokine-1 (MIC-1) (6,7,9,10), or by binding to the mRNAs of p63, murine double minute-2 (MDM2) and p53 and mediating the decrease in the mRNA levels and the attenuation of the translation of these proteins (11-13).

RNPC1 was originally recognized as an oncogene, and was frequently found to be amplified in prostate (14,15), ovarian cancer (16), colorectal cancer (17,18), chronic lymphocytic leukemia (19), colon carcinoma (20), esophageal adenocarcinoma (21), dog lymphomas (13) and breast cancer (22-24). Recently, new evidence suggests that RNPC1 acts as a tumor suppressor. It has been reported that RNPC1 is part of a negative feedback loop, which restricts E2F transcription factor 1 (E2F1) activity by limiting cell cycle progression at the G1-S boundary (25). The expression of RNPC1 has been shown to highly correlate with increased survival in patients with ovarian cancer (25). In breast cancer, RNPC1 functions as a tumor repressor, possibly through promoter hypermethylation silencing (26). In the present study, we identified RNPC1 genes from mammalian genomes using comparative genomic analyses. We then searched for conserved transcription factor-binding sites within the promoter regions of the human RNPC1 gene. Analysis of the expression data and functionally relevant single nucleotide polymorphisms (SNPs), and comparative proteomic analyses were conducted. Furthermore, a meta-analysis of the prognostic value of the RNPC1 gene in various types of cancer was also performed.

## Materials and methods

*Identification of the complete RNPC1 gene in vertebrate genomes and integrative genomic analyses*. The RNPC1 gene and amino acid sequences were selected from the Ensembl database (http://www.ensembl.org/index.html), based on orthologous and paralogous associations. The selected RNPC1 sequences were applied as queries in order to search for the RNPC1 gene using the BLAST tool at the National Center for Biotechnology Information (NCBI), in order to confirm whether their best hit was an RNPC1 gene (27-33). The number, length and structure of the exons and introns in the RNPC1 gene in all species were collected from Ensembl. The number and length of the RNPC1 exons and introns in all sequences were then subjected to exon-intron conservation analyses. Conserved transcription factor-binding sites within the promoter region of the human RNPC1 gene were obtained from the SABiosciences' proprietary database, which combines Text Mining Application and data from the UCSC Genome Browser (http://genome.ucsc.edu/) (27-33).

*Comparative proteomic analyses of RNPC1 protein*. The protein-coding sequences of RNPC1 were aligned using the ClustalW program in MEGA 5.05. We constructed a maximum likelihood (ML) tree of RNPC1 amino acid sequences using MEGA 5.05 with the optimal model (Kimura 2-parameter). Relative support of the internal node was performed by bootstrap analyses with 1,000 replications for ML reconstructions (34). The CodeML program, implemented in the PAML 4.7 software package, was used to investigate whether the RNPC1 protein is under positive selection (35). The site-specific model was developed using the likelihood ratio test (LRT) to compare the M7 (null model) with the M8 model. M7 is a null model that does not allow for any codons with $\omega>1$, whereas the M8 model allows for positively selected sites ($\omega>1$). When the M8 model fits the data significantly (P-value <0.05) better than the null model (M7), the presence of sites with $\omega>1$ is suggested. On the contrary, the results of P-value >0.05 are proof the absence of sites with $\omega>1$. Twice the log likelihood difference between the two compared models ($2\Delta l$) is compared against $\chi^2$ with critical values being 5.99 and 9.21 at the 0.05 and 0.01 significance levels, respectively (36).

*Identification of functionally relevant SNPs in the human RNPC1 gene and somatic mutations in human cancer*. Functionally relevant SNPs of the human RNPC1 gene were identified as previously described (27-33). The SNPs were extracted from Ensembl (http://www.ensembl.org) and NCBI's SNPdb (http://www.ncbi.nlm.nih.gov). The SNPs that disrupted exonic splicing enhancer (ESE)/exonic splicing silencer (ESS) motifs and caused missence mutations were also identified. The identification of somatic mutations of the human RNPC1 gene in human cancer was conducted using COSMIC, a database for mining complete cancer genomes in the catalogue of somatic mutations in cancer (37).

*Analysis of the expression of the human RNPC1 gene*. The expression profiles of RNPC1 in normal human tissues were obtained from ArrayExpress (38). Virtual northern blot analysis of NCBI's UniGene dataset was also performed, as previously described (31-33).

*Meta-analysis of the prognostic value of the RNPC1 gene in cancer*. For meta-analysis, the PrognoScan database was used (39). This includes: i) a large collection of publicly available cancer microarray datasets with clinical annotation, and ii) a tool for assessing the biological association between gene expression and prognosis. PrognoScan employs the minimum P-value approach to group patients for survival analysis. PrognoScan provides a powerful platform for evaluating potential tumor markers and therapeutic targets, and is publicly accessible at http://www.prognoscan.org/. The human RNPC1 gene was inputted as a query, and the data were collected for analysis. PrognoScan displays a summary in table format of tests for RNPC1 with columns for dataset, cancer type, subtype, endpoint, cohort, contributor, array type, probe ID, number of patients, optimal cutpoint, Pmin and Pcor.

## Results

*Comparative proteomic analysis of the RNPC1 protein identified in vertebrate genomes*. All the RNPC1 nucleotide and protein sequences were collected from ENSEMBL and checked using BLAST at NCBI. The complete RNPC1 gene was identified in the human, bushbaby, chimpanzee, macaque, gorilla, olive baboon, vervet-AGM (vervet monkey), guinea pig, mouse, rat, cow, dog, ferret, hedgehog, armadillo, elephant, lesser hedgehog tenrec, anole lizard, chicken, Chinese softshell turtle, duck, Amazon molly, flycatcher, cave fish, Fugu, medaka, platyfish, spotted gar, stickleback, tilapia, *Tetraodon* and zebrafish genomes. The sequences and structural alignment of RNPC1 in these genomes are shown in Fig. 1. The phylogenetic tree

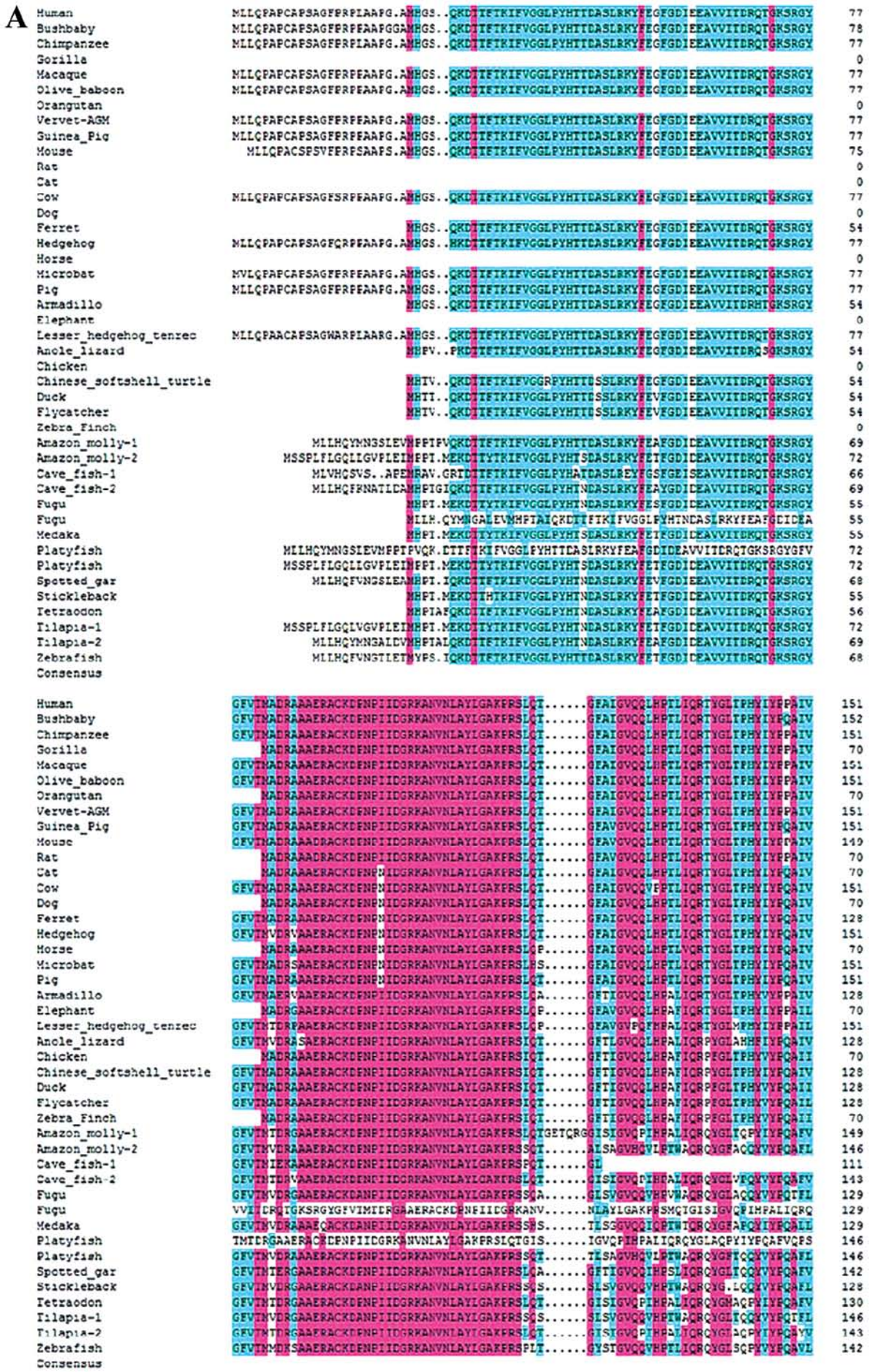


Figure 1. Sequence and structural alignment of RNPC1 in vertebrates. (A) Alignment of RNPC1 in vertebrates from position 1-151.

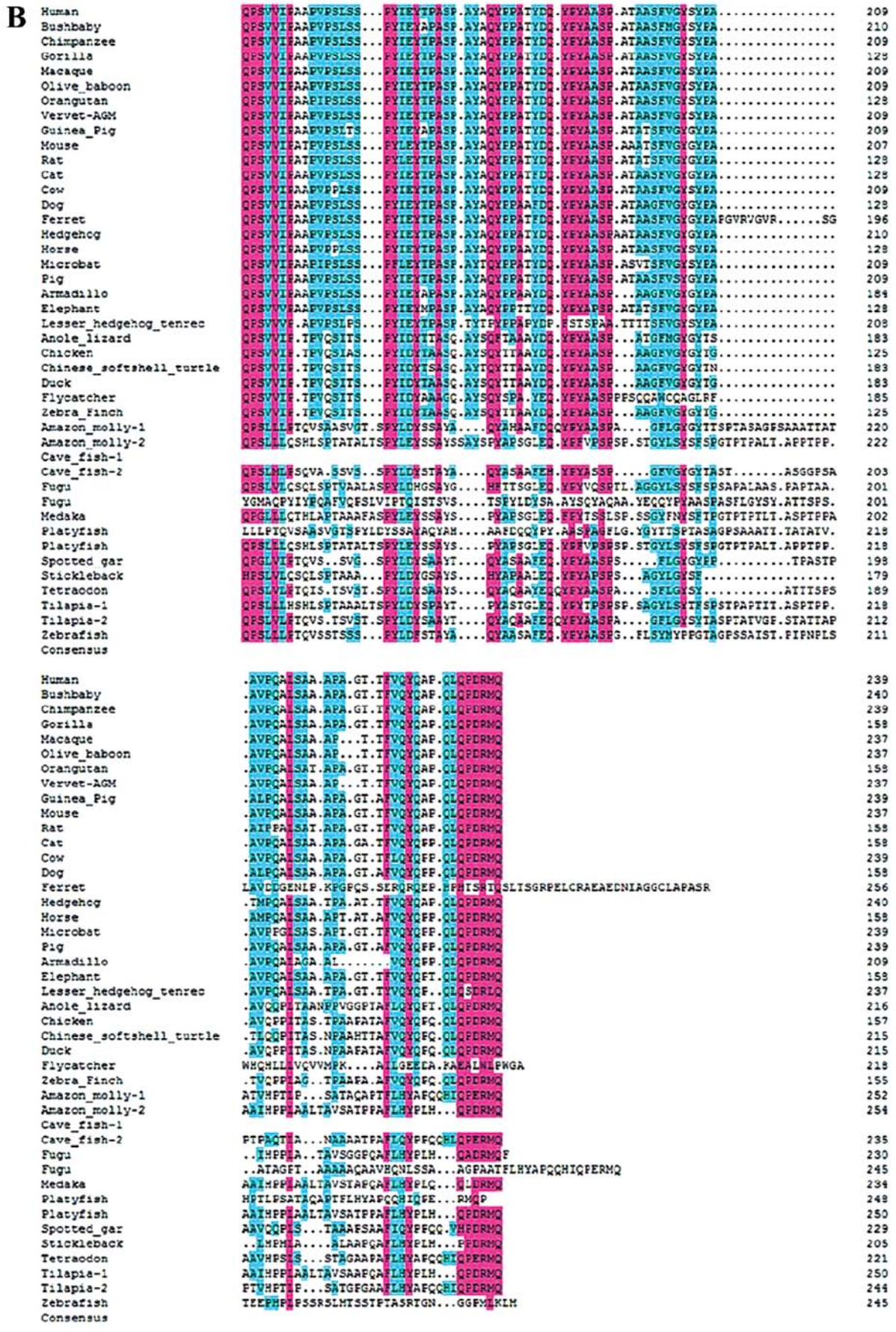


Figure 1. Continued. (B) Alignment of RNPC1 in vertebrates from position 152-239. All the RNPC1 gene and protein sequences were collected from Ensembl and checked using the BLAST tool at NCBI. The complete RNPC1 gene was identified in the human, bushbaby, chimpanzee, macaque, gorilla, olive baboon, vervet-AGM, guinea pig, mouse, rat, cow, dog, ferret, hedgehog, armadillo, elephant, lesser hedgehog tenrec, anole lizard, chicken, Chinese softshell turtle, duck, Amazon molly, flycatcher, cave fish, fugu, medaka, platyfish, spotted gar, stickleback, tilapia, *tetraodon* and zebrafish genomes.

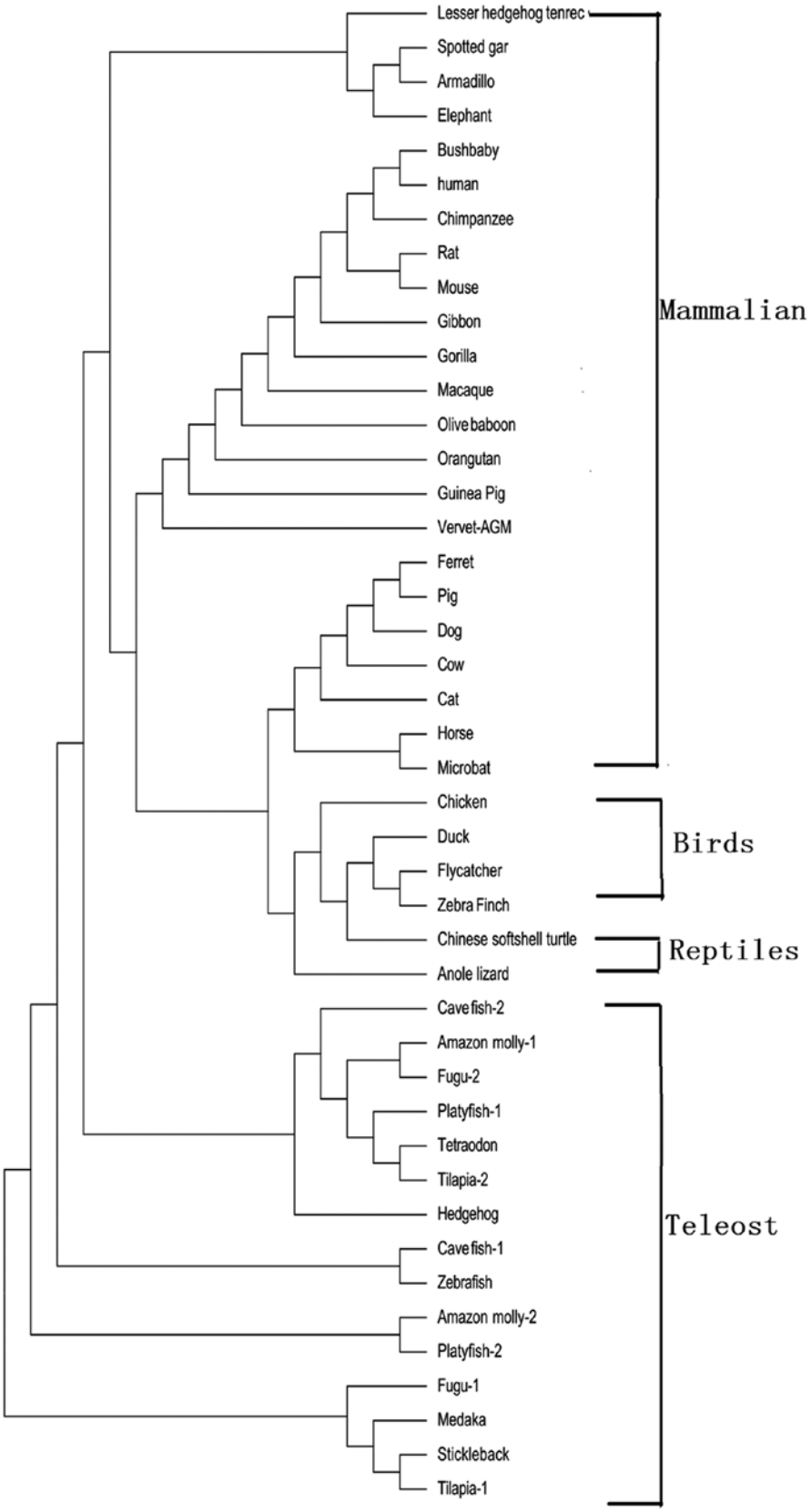


Figure 2. The phylogenetic tree was constructed according to the protein-coding sequences of RNPC1 using the maximum likelihood method. The RNPC1 gene from the mammalian, bird, reptile and teleost lineages formed species-specific clusters.

was constructed according to the protein-coding sequences of RNPC1, using the maximum likelihood method; the RNPC1 gene from the mammalian, bird, reptile and teleost lineages formed species-specific clusters (Fig. 2). The exon-intron data

Table I. Exon and intron lengths of RNPC1.

| Species | Exon 1 | Intron 1 | Exon 2 | Intron 2 | Exon 3 | Intron 3 | Exon 4 | Intron 4 | Exon 5 | Total exons |
|---|---|---|---|---|---|---|---|---|---|---|
| Human | 237 | 835 | 124 | 501 | 55 | 14209 | 304 | | | 720 |
| Bushbaby | 240 | 842 | 124 | 430 | 55 | 11713 | 304 | | | 723 |
| Chimpanzee | 720 | | | | | | | | | 720 |
| Macaque | 237 | 816 | 124 | 503 | 55 | 13671 | 298 | | | 714 |
| Olive baboon | 237 | 835 | 124 | 508 | 55 | 13677 | 298 | | | 714 |
| Vervet-AGM | 237 | 841 | 124 | 505 | 55 | 13836 | 298 | | | 714 |
| Mouse | 0 | 1110 | 231 | 521 | 124 | 411 | 55 | 10124 | 304 | 714 |
| Rat | 118 | 489 | 55 | 9816 | 300 | | | | | 473 |
| Guinea Pig | 237 | 748 | 124 | 442 | 55 | 10426 | 301 | | | 717 |
| Cow | 237 | 753 | 124 | 483 | 55 | 12228 | 304 | | | 720 |
| Pig | 237 | 778 | 124 | 559 | 55 | 11296 | 304 | | | 720 |
| Armadillo | 168 | 747 | 124 | 399 | 55 | 12317 | 283 | | | 630 |
| Amazon molly-1 | 213 | 4109 | 141 | 1707 | 56 | 6429 | 349 | | | 759 |
| Amazon molly-2 | 222 | 4039 | 124 | 2832 | 55 | 13455 | 364 | | | 765 |
| Cave fish-2 | 213 | 2209 | 124 | 151 | 55 | 4745 | 316 | | | 708 |
| Fugu-1 | 171 | 789 | 124 | 1405 | 55 | 5561 | 340 | | | 690 |
| Medaka | 171 | 2679 | 124 | 2251 | 55 | 13729 | 355 | | | 705 |
| Platyfish-2 | 222 | 4066 | 124 | 2973 | 55 | 15548 | 352 | | | 753 |
| Spotted gar | 210 | 2827 | 124 | 2302 | 55 | 6210 | 301 | | | 690 |
| Stickleback | 171 | 904 | 124 | 1223 | 55 | 6070 | 187 | 54 | 81 | 618 |
| *Tetraodon* | 174 | 1629 | 124 | 269 | 55 | 3386 | 214 | 27 | 99 | 666 |
| Zebrafish | 211 | 13848 | 124 | 12313 | 55 | 17086 | 247 | 4 | 102 | 739 |

collected from the ENSEMBL database are shown in Table I and Fig. 3. In the majority of vertebrates, the RNPC1 gene exhibited exon-intron conservation, with 4 exons and 3 introns, with similar sizes for each exon and intron (Table I). However, there were 5 exons and 4 introns in the RNPC1 gene in the mouse and 3 fish species (stickleback, *tetraodon* and zebrafish). Thus, the intron deletions in the RNPC1 gene may occur during the evolutionary process of these 3 species of fish. Furthermore, site-specific tests for positive selection were performed for the vertebrate, mammalian, primate, and mammalian excluding primate, rodent and teleost lineages. We were unable to identify any site which was under positive selection by the M7 and M8 models in the RNPC1 protein. It seemed that RNPC1 in vertebrates was under purifying selection (data not shown).

*Expression profile of the human RNPC1 gene*. The investigation of the available microarray data and virtual northern blot analysis, we revealed the predominant expression of RNPC1 in bone marrow, whole blood, lymph node, thymus, brain, cerebellum, retina, spinal cord, heart, smooth muscle, skeletal muscle, small intestine, colon, adipocyte, kidneys, liver, lungs, pancreas, thyroid, salivary gland, skin, breast, ovaries, uterus, placenta, prostate and testes. When we searched the PrognoScan database, we found that human RNPC1 was also expressed in bladder, blood, brain, breast, colorectal, eye, head and neck, lung, ovarian, skin and soft tissue cancer.

*Comparative genomic analysis of the human RNPC1 gene*. The sex determining region Y (SRY)-box 5 (Sox5), runt-related transcription factor 3 (RUNX3), CCAAT displacement protein 1 (CUTL1), v-rel avian reticuloendotheliosis viral oncogene homolog (Rel)A, peroxisome proliferator-activated receptor γ isoform 2 (PPARγ2) and activating transcription factor 6 (ATF6) regulatory transcription factor binding sites were identified in the upstream (promoter) region of the RNPC1 gene.

*Identification of functionally relevant SNPs in the human RNPC1 gene and somatic mutations in human cancer.* A total of 429 SNPs were identified in the human RNPC1 gene. Of these, 34 SNPs were functionally relevant, including 14 SNPs causing missense mutations, 8 exonic splicing enhancer SNPs and 12 SNPs causing nonsense mutations (Table II). By searching the COSMIC database, we identified 30 somatic mutations of RNPC1 in 10,148 cancer samples (Table III).

*Meta-analysis of the prognostic value of the human RNPC1 gene in cancer.* When provided with the specific gene, PrognoScan displays a summary (in table format) of tests for the gene, with columns for the dataset, cancer type, subtype, endpoint, cohort, contributor, array type, probe ID, number of patients, optimal cut-point, Pmin and Pcor. Among the databases which detected the expression of the RNPC1 gene, an association between the expression of the RNPC1 gene and cancer prognosis was noted in 14 of the 94 tests (blood cancer 2/9, brain cancer 1/5, breast cancer 3/30, colorectal cancer 1/9, eye 1/1, head and neck cancer 0/1, lung cancer 5/24, ovarian cancer 1/10, skin cancer 0/1 and soft tissue cancer 0/1),

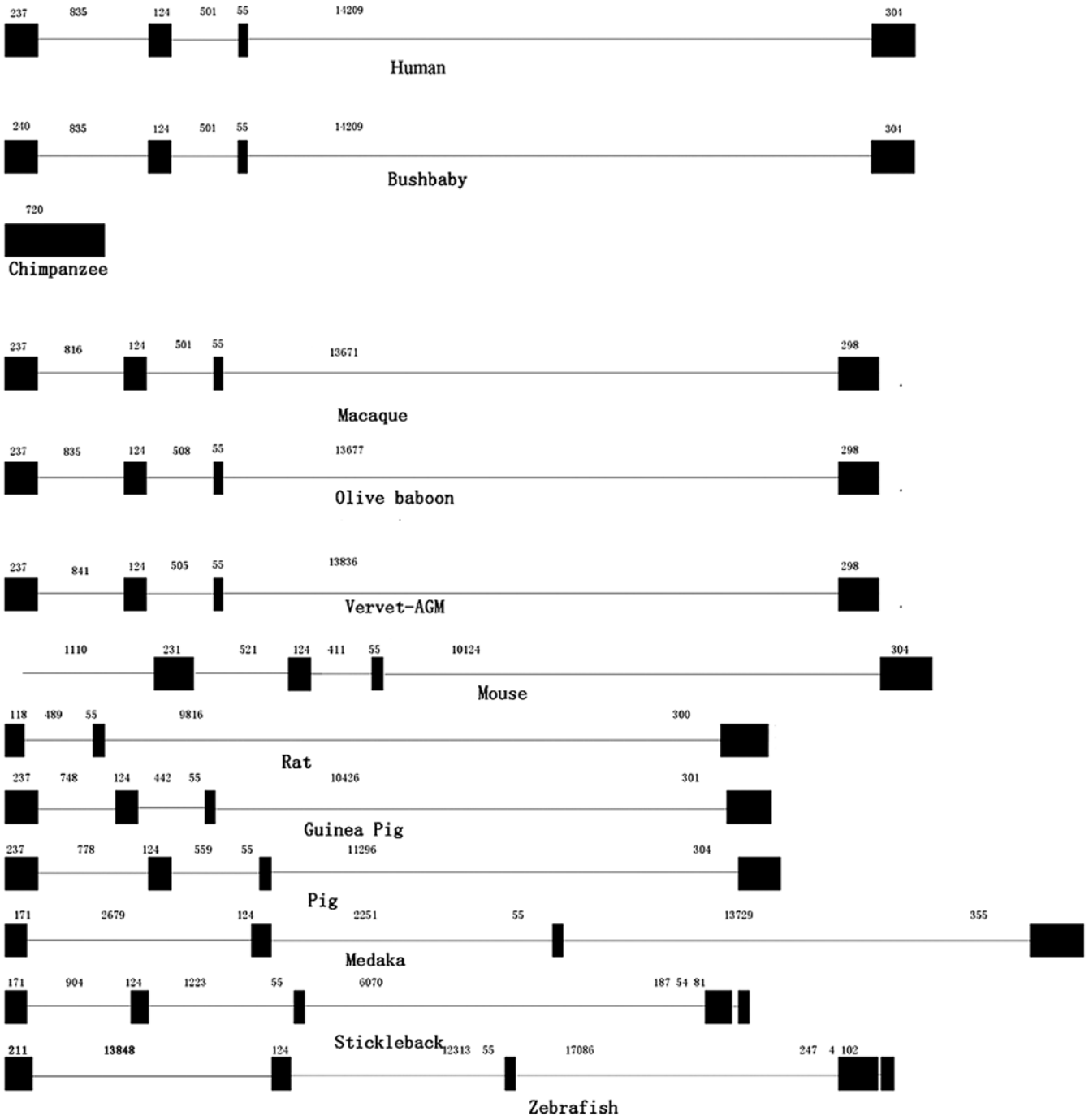


Figure 3. Exon-intron conservation among the RNPC1 gene. In the majority of vertebrates, the RNPC1 gene showed a similar exon-intron conservation, namely 4 exons and 3 introns, with similar sizes for each exon and intron (Table I). However, there are 5 exons and 4 introns in the RNPC1 gene in the mouse and 3 fish species (stickleback, *tetraodon* and zebrafish).

with a 5% significance level (Table IV). As regards blood, colorectal and eye cancer, a correlation between the decreased expressino of the RNPC1 gene and poor survival was observed. However, a higher expression of the RNPC1 gene was found to correlated with a poor survival in patients with brain and ovarian cancer. Of the 3 breast cancer cases, a lower expression of the RNPC1 gene, which correlated with poor survival, was observed in 2 cases (E-TABM-158 and GSE7849), while a higher expression of the RNPC1 gene correlated with a poor survival in the case of GSE11121. Of the lung cancer cases, a lower expression of the RNPC1 gene, which correlated with poor survival, was noted in 2 cases (GSE31210 and GSE31211), while a higher expression of the RNPC1 gene correlated with poor survival in 3 cases (HARVARD-LC, GSE4716-GPL3694 and Jacob-00182-CANDF) cases.

## Discussion

RNPC1 (also known as RBM38), is an RBP that contains one RRM domain. It is expressed as two isoforms, RNPC1a and

Table II. Functionally relevant SNPs in the human RNPC1 gene.

| SNP ID | Chr 20 position sequence | Sequence | Type | Amino acid change |
|---|---|---|---|---|
| rs150246007 | 55982673(+) | CCCGTC/TGCTGT | mis | S L |
| rs201278266 | 55982720(+) | CCTACA/GCCCAG | mis | T A |
| rs201875738 | 55982772(+) | GCCTGC/TCACGG | mis | A V |
| rs199521379 | 55982775(+) | TGCCAC/TGGCTG | mis | T M |
| rs201066490 | 55982813(+) | CCGCCA/GTGCCC | mis | M V |
| rs201744631 | 55982843(+) | CACCCA/GCGGGC | mis | T A |
| rs16980970 | 55982858(+) | CTTTCG/CTGCAG | mis | L V |
| rs1065289 | 55982781(+) | GGCTGC/ACAGCT | mis | D A |
| rs369246420 | 55982844(+) | ACCCGC/TGGGCA | mis | A V |
| rs373452137 | 55982615(+) | ACTACA/GTCTAC | mis | I V |
| rs377081682 | 55982714(+) | GCCCGG/TCCTAC | mis | A S |
| rs368322258 | 55982789(+) | GCTTCA/GTGGGC | mis | M V |
| rs10652881 | 55982715(+) | CCCGGC/TCTACG | mis | A V |
| rs1065290 | 55982817(+) | CGTGCC/ACCAGG | mis | H P |
| rs11546710 | 55983073(+) | AGAGAC/TGGCTT | ese | |
| rs6128022 | 55983476(+) | TCCCAG/AGCGCA | ese | |
| rs8126441 | 55983505(+) | GGGGCC/AGCCGG | ese | |
| rs3829703 | 55983509(-) | TTGGCC/TGGCGG | ese | |
| rs11546713 | 55984212(-) | CCCCCA/GCCCTC | ese | |
| rs1065292 | 55983556(+) | TTTTTC/TTTGTA | ese | |
| rs1052752 | 55983512(+) | CCGGCC/AAAAGG | ese | |
| rs3207621 | 55983521(+) | GGCCCC/TTTTCC | ese | |
| rs141028132 | 55982671(+) | GTCCCG/ATCGCT | syn | P |
| rs201839752 | 55982683(+) | TCCTCA/GCCCTA | syn | S |
| rs115516069 | 55982695(+) | ATTGAG/ATACAC | syn | E |
| rs200910302 | 55982719(+) | GCCTAC/TGCCCA | syn | Y |
| rs199953546 | 55982758(+) | CCATAC/TGCCGC | syn | Y |
| rs143107197 | 55982812(+) | GCCGCC/TGTGCC | syn | A |
| rs373297597 | 55982875(+) | GCGCCA/GCAGCT | syn | P |
| rs373492567 | 55982887(+) | CAGCCA/TGACAG | syn | P |
| rs202004284 | 55982704(+) | ACGCCG/AGCCAG | syn | P |
| rs376442730 | 55982872(+) | CAGGCA/GCCGCA | syn | A |
| rs377524807 | 55982644(+) | CCCAGC/TGTGGT | syn | S |
| rs374582705 | 55982713(+) | AGCCCA/GGCCTA | syn | P |

A total of 429 single nucleotide polymorphisms (SNPs) were identified in the human RNPC1 gene. Of these, 34 SNPs were functionally relevant, including 14 SNPs causing missense (mis) mutations, 8 exonic splicing enhancer (ese) SNPs and 12 SNPs causing nonsense mutations. Chr, chromosome.

RNPC1b (6). RNPC1 is a direct target of p53 and can interact with other members of the p53 family; it can stabilize p21 and p73 transcripts and destabilize p63 transcripts. It can also bind and stabilize the mRNA of the CDK inhibitor, p21, thereby inducing cell cycle arrest in the G1 phase (7,8). RNPC1 also binds and stabilizes the mRNA of another RBP HuR, which in turn facilitates RNPC1-mediated growth arrest (7). In the present study, the complete RNPC1 gene was identified in the human, bushbaby, chimpanzee, macaque, gorilla, olive baboon, vervet-AGM, guinea pig, mouse, rat, cow, dog, ferret, hedgehog, armadillo, elephant, lesser hedgehog tenrec, anole lizard, chicken, Chinese softshell turtle, duck, Amazon molly, flycatcher, cave fish, Fugu, medaka, platyfish, spotted gar, stickleback, tilapia, *tetraodon* and zebrafish genomes, suggesting that RNPC1 exists in all types of vertebrates, including fish, amphibians, birds and mammals. In the different genomes, the gene had a similar organization, namely 4 exons/3 introns, and all the genetic loci were syntenically conserved. The phylogenetic tree revealed that the RNPC1 gene from the mammalian, bird, reptile and teleost lineage formed species-specific clusters. As observed from the alignment and phylogenetic tree, RNPC1 in mammals is conserved among vertebrate genomes,

Table III. Somatic mutations of RNPC1 in cancer tissue.

| Position (AA) | Mutation (CDS) | Mutation (amino acid) | Mutation ID (COSM) | Count | Mutation type |
|---|---|---|---|---|---|
| 19 | c.55G>C | p.A19P | COSM1412673 | 2 | Substitution - missense |
| 20 | c.56_57insC | p.A20fs*70 | COSM3724433 | 2 | Insertion - frameshift |
| 49 | c.146C>G | p.S49W | COSM3963694 | 1 | Substitution - missense |
| 55 | c.163G>C | p.E55Q | COSM397137 | 1 | Substitution - missense |
| 78 | c.234C>T | p.G78G | COSM724075 | 1 | Substitution - coding silent |
| 83 | c.249C>T | p.A83A | COSM1165242 | 1 | Substitution - coding silent |
| 89 | c.265G>A | p.E89K | COSM724074 | 1 | Substitution - missense |
| 89 | c.266A>G | p.E89G | COSM117504 | 1 | Substitution - missense |
| 97 | c.290C>T | p.P97L | COSM224597 | 1 | Substitution - missense |
| 109 | c.325G>A | p.A109T | COSM1412674 | 1 | Substitution - missense |
| 112 | c.336C>T | p.G112G | COSM192074 | 1 | Substitution - coding silent |
| 116 | c.346C>T | p.R116W | COSM1579588 | 1 | Substitution - missense |
| 116 | c.347G>A | p.R116Q | COSM724073 | 1 | Substitution - missense |
| 120 | c.359C>T | p.T120M | COSM1028363 | 1 | Substitution - missense |
| 131 | c.392C>G | p.P131R | COSM3405224 | 1 | Substitution - missense |
| 132 | c.395C>G | p.T132S | COSM3363328 | 1 | Substitution - missense |
| 139 | c.415G>A | p.G139R | COSM125790 | 1 | Substitution - missense |
| 147 | c.441A>C | p.P147P | COSM4134683 | 1 | Substitution - coding silent |
| 150 | c.450C>T | p.I150I | COSM1565701 | 1 | Substitution - coding silent |
| 160 | c.478G>A | p.A160T | COSM1412675 | 1 | Substitution - missense |
| 163 | c.487C>T | p.P163S | COSM3548089 | 1 | Substitution - missense |
| 168 | c.502C>T | p.P168S | COSM3548090 | 1 | Substitution - missense |
| 172 | c.515A>G | p.Y172C | COSM4099709 | 1 | Substitution - missense |
| 174 | c.521C>T | p.P174L | COSM1412676 | 1 | Substitution - missense |
| 176 | c.527G>A | p.S176N | COSM270012 | 1 | Substitution - missense |
| 193 | c.577G>A | p.A193T | COSM3770849 | 1 | Substitution - missense |
| 193 | c.579C>T | p.A193A | COSM3548091 | 1 | Substitution - coding silent |
| 210 | c.628G>A | p.A210T | COSM4099710 | 1 | Substitution - missense |
| 220 | c.659C>T | p.P220L | COSM3911642 | 1 | Substitution - missense |
| 225 | c.675C>T | p.F225F | COSM263280 | 1 | Substitution - coding silent |

suggesting that the function of RNPC1 plays an important physiological role in all vertebrates during the evolution process.

The investigation of available microarray data and virtual northern blot analysis confirmed the predominant expression of RNPC1 in the bone marrow, whole blood, the lymph node, thymus, brain, cerebellum, retina, spinal cord, heart, smooth muscle, skeletal muscle, small intestine, colon, adipocyte, kidneys, liver, lungs, pancreas, thyroid, salivary gland, skin, breast, ovaries, uterus, placenta, prostate and testes. Thus, RNPC1 is widely expressed in a number of tissues and organs. A total of 429 SNPs were identified in the human RNPC1 gene. Of these, 34 SNPs were functionally relevant, including 14 SNPs causing missense mutations, 8 exonic splicing enhancer SNPs and 12 SNPs causing nonsense mutations, which may affect the multiple functions of RNPC1. However, the effects of these SNPs on RNPC1 physiological and pathological functions require further investigation.

RNPC1 was originally recognized as an oncogene, and was frequently found to be amplified in prostate (14,15), ovarian (16) and colorectal cancer (17,18), chronic lymphocytic leukemia (19), colon carcinoma (20), esophageal cancer (21), dog lymphomas (13) and breast cancer (22-24). In our previous study, we found that RNPC1 played a tumor suppressor role role in breast cancer (40). In the present study, we first noted that RNPC1 was indeed expressed in bladder, blood, brain, breast, colorectal, eye, head and neck, lung, ovarian, skin and soft tissue cancer. Out of 94 tests, 14 revealed an association between RNPC1 gene expression and cancer prognosis (blood 2/9, brain 1/5, breast 3/30, colorectal 1/9, eye 1/1, head and neck 0/1, lung 5/24, ovarian 1/10, skin 0/1 and soft tissue cancer 0/1). It is important to note that the association between the expression of RNPC1 and prognosis varied in different types of cancer, and even in the same type of cancer from different databases. This suggests that the function of RNPC1 in these tumors may be multidimensional, and that RNPC1 is

Table IV. Dataset content from PrognoScan demonstrating an association between the expression of the RNPC1 gene and cancer prognosis.

| Database | Case type | Subsyte | No. of patients | Endpoint | Cut-point | P-value | Prognosis | Refs. |
|---|---|---|---|---|---|---|---|---|
| GSE12417-GPL570 | Blood cancer | AML | 79 | Overall survival | 0.15 | 0.001223 | 1 | (57) |
| GSE12417-GPL96 | Blood cancer | AML | 163 | Overall survival | 0.39 | 0.035113 | 1 | (57) |
| GSE4271-GPL96 | Brain cancer | Astrocytoma | 77 | Overall survival | 0.82 | 0.003856 | 2 | (58) |
| GSE7849 | Breast cancer | | 76 | Disease-free survival | 0.3 | 0.030908 | 1 | (59) |
| GSE11121 | Breast cancer | | 200 | Distant metastasis-free survival | 0.82 | 0.008577 | 2 | (60) |
| E-TABM-158 | Breast cancer | | 117 | Disease-specific survival | 0.7 | 0.035878 | 1 | (61) |
| GSE17537 | Colorectal cancer | | 55 | Overall survival | 0.11 | 0.02856 | 1 | (62) |
| GSE22138 | Eye cancer | Uveal melanoma | 63 | Distant metastasis-free survival | 0.33 | 0.018941 | 1 | (63) |
| jacob-00182-CANDF | Lung cancer | Adenocarcinoma | 82 | Overall survival | 0.82 | 0.020135 | 2 | (64) |
| HARVARD-LC | Lung cancer | Adenocarcinoma | 84 | Overall survival | 0.69 | 0.004177 | 2 | (65) |
| GSE31210 | Lung cancer | Adenocarcinoma | 204 | Relapse-free survival | 0.51 | 0.001557 | 1 | (66) |
| GSE31211 | Lung cancer | Adenocarcinoma | 204 | Overall survival | 0.34 | 0.000939 | 1 | (66) |
| GSE4716-GPL3694 | Lung cancer | NSCLC | 50 | Overall survival | 0.82 | 0.044441 | 2 | (67) |
| DUKE-OC | Ovarian cancer | | 133 | Overall survival | 0.85 | 0.010709 | 2 | (68) |

Of the 94 tests, in 14, we noted an association between the expression of the RNPC1 gene and cancer prognosis (blood cancer 2/9, brain cancer 1/5, breast cancer 3/30, colorectal cancer 1/9, eye cancer 1/1, head and neck cancer 0/1, lung cancer 5/24, ovarian cancer 1/10, skin cancer 0/1 and soft tissue cancer 0/1) with a 5% significance level. AML, acute myeloid leukemia; NSCLC, non-small cell lung cancer.

not just a tumor suppressor or promoter. Moreover, we identified 30 somatic mutations of RNPC1 in cancer tissues in the present study. Further investigation is required to elucidate the mechanisms through which these mutations affect tumor formation. The mechanisms underlying the role of RNPC1 in the process of these tumors may be involve the mRNA stabilizion of oncogenes or anti-oncogenes, such as p53 (13), p63 (11), MDM2 (12), p73 (9), HuR (7) and p21 (6). However, the mechanisms underlying the role of RNPC1 in the developmental process of these tumors require further investigation.

The Sox5, RUNX3, CUTL1, RelA, CCAAT-enhancer-binding protein (C/EBP)α, c-Ets-1, PPARγ2 and ATF6 regulatory transcription factor binding sites were identified in the upstream (promoter) region of the RNPC1 gene. Sox5 plays a role in the regulation of embryonic development and in the determination of cell fate. It can function as a transcriptional regulator after forming a protein complex with other proteins. It has a negative effect on cell proliferation in some cell types and functions as a target of microRNAs (41,42). RUNX3 encodes a member of the runt domain-containing family of transcription factors. A heterodimer of this protein and a β subunit forms a complex that binds to the core DNA sequence 5'-PYGPYGGT-3' found in a number of enhancers and promoters, and can either activate or suppress transcription. It functions as a tumor suppressor and is frequently deleted or transcriptionally silenced in cancer (43-46). CUTL1 is a transcription factor which plays a role in development and multiple physiological processes. Emerging evidence indicates that CUTL1 is not only involved in developmental events, but also in pathological processes, such as tumorigenesis and multiple signal transduction pathways of cancer (47,48). RelA is a subunit of the nuclear factor (NF-κB) p65. NF-κB is an ubiquitous transcription factor which plays a role in several biological processes. NF-κB is composed of NFKB1 or NFKB2 bound to either REL, RELA or RELB. NF-κB is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to a number of biological processes, such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis (49-52). C/EBPα is required for the proper control of adipogenesis, glucose metabolism, granulocytic differentiation, lung development and the development of various types of cancer (53,54). c-Ets-1 is known to play an important role in various biological processes, such as development, differentiation, proliferation, apoptosis, migration, tissue remodeling, invasion and angiogenesis in a variety of cell types, including B cells, endothelial cells, fibroblasts and neoplastic cells (55,56). These tumor-related transcriptional factors may be involved in the effects of RNPC1 in tumors (14-24).

In conclusion, integrative genomic analyses of RNPC1 and its role in cancer prediction provide a powerful tool for the evaluation of RNPC1 as a potential tumor markers and therapeutic targets in cancer research.

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