Prognostic value of ATAD3 gene cluster expression in hepatocellular carcinoma

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Abstract. ATPase family AAA domain-containing protein 3 (ATAD3) is a mitochondrial membrane-bound ATPase that is involved in a number of cellular processes and is linked with the progression of various types of malignancies. In primates, the ATAD3 gene cluster contains ATAD3A, ATAD3B and ATAD3C. The association between ATAD3 gene cluster expression and hepatocellular carcinoma (HCC) remains unknown. Therefore, the present study examined the prognostic significance of ATAD3 gene cluster expression in patients with HCC. Box plots of expression differences between HCC and normal liver tissues for the ATAD3 family genes were obtained from the online tool Gene Expression Profiling Interactive Analysis. Data from 360 patients with HCC in The Cancer Genome Atlas database were analyzed. Kaplan-Meier analysis and a Cox regression model were used to calculate median survival time (MST) and overall survival (OS). ATAD3A and ATAD3B expression levels were higher in HCC compared with normal liver tissues (P<0.05). However, ATAD3C expression was significantly decreased in HCC tissues compared with normal liver tissues (P<0.05). ATAD3A [P=0.017, hazard ratio (HR)=1.54, 95% confidence interval (CI)=1.08-2.20; adjusted P=0.032; adjusted HR=1.52; 95% CI=1.04-2.22] and ATAD3B (P=0.026, HR=1.49, 95% CI=1.05-2.13; adjusted P=0.031, adjusted HR=1.52, 95% CI=1.04-2.21) expression levels were significantly associated with OS. A joint-effects analysis revealed that patients with high ATAD3A and ATAD3B expression had reduced OS rates compared with patients with low ATAD3A and ATAD3B expression (P=0.007, HR=1.77, 95% CI=1.16-2.69; adjusted P=0.013, adjusted HR=1.76, 95% CI=1.13-2.75). In conclusion, ATAD3A and ATAD3B may serve as potential prognostic biomarkers for patients with HCC.

Introduction

Liver cancer is one of the most prevalent types of malignancy worldwide as the annual estimated rate reached 782,500 novel cases and ~745,500 liver cancer-associated mortalities in 2012 (1). In China, patients account for ~50% of all these cases and mortalities (1). Hepatocellular carcinoma (HCC) is the major histologic liver cancer subtype, representing 80% of all liver malignancies (2). Although surgical resection and liver transplantation are used in the treatment of early-stage HCC, the overall prognosis remains poor, due to high recurrence rates (3). A number of studies have reported that gene expression signatures are associated with the prognosis of HCC (4-6). Therefore, identifying novel biomarkers that predict prognosis and may guide individualized treatment for HCC would greatly benefit patients.

ATPase family AAA domain-containing protein 3 (ATAD3) is a mitochondrial membrane-bound ATPase that was first identified as a component of the mouse liver inner mitochondrial membrane using a proteomic approach (7) and was subsequently discovered to be overexpressed in head and neck carcinomas (8). Subsequent studies have reported that ATAD3 serves important roles in Caenorhabditis elegans and Drosophila melanogaster development, indicating that ATAD3 is associated with proliferation and differentiation (9,10). In primates, the ATAD3 gene cluster contains ATAD3A, ATAD3B and ATAD3C. ATAD3A is the ancestral form of ATAD3, while ATAD3B and ATAD3C are similar, however, they contain important mutated residues (11). These three genes are located side-by-side at the end of chromosome 1 (locus 1p36.33).

ATAD3 is a member of the family of AAA-ATPases, which are involved in a number of cellular processes, including transcription, replication, translation, proteolysis, and vesicular transport (12). In HeLa cells, ATAD3 was reported in a large multi-molecular complex associated with mitochondrial DNA (mtDNA) that serves a role in mtDNA replication and transcription (13). ATAD3 protein has displacement loop binding activity, which allows it to form or segregate mitochondrial
nucleoids (14). However, Bogenhagen et al (15) have reported that ATAD3A and ATAD3B indirectly interact with mtDNA, mediated by topology rather than the C-terminal AAA domain. Therefore, they do not have the opportunity to bind to mtDNA D-loops. Subsequent results have demonstrated that ATAD3A controls mitochondrial dynamics between the outer and inner membranes, and that the N-terminal region of ATAD3A is outside the inner membrane, while the C-terminal region is within the matrix (16,17). ATAD3 deficiency is associated with aberrant mtDNA organization and cholesterol metabolism in the central nervous system (18).

ATAD3 expression was originally reported to produce autoimmune responses in patients with lung adenocarcinoma or uterine cervical cancer (19,20) and to be associated with tumorigenesis (11). Studies have reported that ATAD3 expression is linked with the progression of head and neck cancers (8), non-Hodgkin’s lymphoma (21), lung cancer (22), uterine cervical cancer (23), prostate cancer (24), and glioma (25). However, to the best of our knowledge, there have been no studies investigating associations between ATAD3 expression and HCC. In the present study, the prognostic value of ATAD3 gene cluster expression was investigated in HCC to determine its potential as a biomarker for this disease.

Materials and methods

ATAD3 gene cluster expression in HCC and normal liver tissues. Box plots comparing expression levels of the ATAD3 gene cluster in HCC (n=369) vs. normal liver tissues (n=50) were downloaded from the online tool Gene Expression Profiling Interactive Analysis (http://geopia.cancer-pku.cn/), which uses data derived from The Cancer Genome Atlas (TCGA; http://tcga-data.nih.gov/tcga). Significance cut-off level was set at P=0.05.

Patient information. Clinical data and ATAD3A, ATAD3B and ATAD3C mRNA levels of the 360 patients were obtained from the online websites OncoLnc (http://www.oncolnc.org/) and TCGA. The present study’s results are partially based on data generated by TCGA Research (http://cancergenome.nih.gov/). The included clinical data were race, sex, age, body mass index (BMI), tumor node metastasis (TNM) stage (the seventh index (BMI), tumor node metastasis (TNM) stage (the seventh stage) (26), survival time (days), and survival status.

Survival analysis. ATAD3A, ATAD3B and ATAD3C mRNA expression levels from TCGA were individually divided into two groups by their 50% cut-off values, resulting into the high-expression (n=180) and low-expression groups (n=180). Overall survival (OS) was analyzed by the Cox proportional hazards regression model adjusted by sex, age, and tumor stage.

Joint-effects analysis. ATAD3A and ATAD3B expression indicated statistically significant associations with OS in the patient cohort with HCC. Therefore, a joint-effects analysis of the combination of ATAD3A and ATAD3B with group I (low ATAD3A and ATAD3B expression), group II (low ATAD3A and high ATAD3B expression), group III (high ATAD3A and low ATAD3B expression), and group IV (high ATAD3A and ATAD3B expression) was performed. Sex, age, and tumor stage were adjusted in the Cox proportional hazards regression model.

Gene co-expression network analysis. In order to predict gene function and to construct a pathway for the ATAD3 genes, the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/content.jsp?file=citation.htm) was used to carry out the enriched Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (27). Cytoscape 3.6.0 software (https://cytoscape.org/) was used to construct biological networks (28).

Results

Analysis of ATAD3 gene cluster expression in HCC and normal liver tissues. Expression data from 369 HCC and 50 normal liver samples were analyzed by box plots, and the results indicated that ATAD3A and ATAD3B were significantly overexpressed in HCC tissues compared with normal liver tissues (P<0.05; Fig. 1). However, the expression level of ATAD3C was significantly reduced in HCC tissues compared with normal liver tissues (P<0.05; Fig. 1).

TCGA database patient characteristics. Clinical characteristics of the 360 patients from the TCGA database are presented in Table I. The cohort included 244 male and 116 female patients, and the median age was 61 years. The analysis indicated that TNM stage was significantly associated with OS (P<0.001; HR=2.50; 95% CI=1.72-3.63), whereas neither race, sex, age nor BMI were associated with OS.

Survival analysis of ATAD3 mRNA levels with OS. ATAD3A, ATAD3B and ATAD3C mRNA expression data were available for all patients from the TCGA database. The patients were divided into two groups based on the 50% cut-off level for each mRNA. The correlations between each gene and OS were analyzed. The results indicated that the expression level of ATAD3A (P=0.017; HR=1.54, 95% CI=1.08-2.20; adjusted P=0.032; adjusted HR=1.52; 95% CI=1.04-2.22) and ATAD3B (P=0.026, HR=1.49, 95% CI=1.05-2.13; adjusted P=0.031, adjusted HR=1.52, 95% CI=1.04-2.21) were significantly correlated with OS (Table II; Fig. 2A and B). ATAD3C expression level was not significantly associated with OS (Table II; Fig. 2C). Furthermore, a joint-effects analysis of ATAD3A and ATAD3B with OS was performed, which demonstrated that patients with high expression levels of both ATAD3A and ATAD3B had a worse OS compared with those with low expression levels of ATAD3A and ATAD3B (P=0.007, HR=1.77,
Table I. Demography and clinical characteristics of 360 patients with hepatocellular carcinoma in The Cancer Genome Atlas database.

| Overall survival | Patients (n=360) | MST (days) | HR (95% CI) | Log-rank P-value |
|------------------|-----------------|------------|-------------|-----------------|
| **Race**         |                 |            |             |                 |
| Asian            | 155             | NA         | 1.29 (0.89-1.87) | 0.188          |
| White+other      | 196             | 1,397      |             |                 |
| Missing          | 9               |            |             |                 |
| **Sex**          |                 |            |             |                 |
| Male             | 244             | 2,486      | 1.21 (0.84-1.73) | 0.311          |
| Female           | 116             | 1,560      |             |                 |
| **Age (years)**  |                 |            |             |                 |
| <61              | 186             | 2,116      | 1.09 (0.77-1.54) | 0.622          |
| ≥61              | 171             | 1,622      |             |                 |
| Missing          | 3               |            |             |                 |
| **BMI**          |                 |            |             |                 |
| ≤25              | 193             | 2,456      | 0.87 (0.60-1.27) | 0.473          |
| >25              | 137             | 2,116      |             |                 |
| Missing          | 30              |            |             |                 |
| **TNM stage**    |                 |            |             |                 |
| I+II             | 252             | 2,532      | 2.50 (1.72-3.63) | <0.001         |
| III+IV           | 87              | 770        |             |                 |
| Missing          | 21              |            |             |                 |

Where survival rate was >50% in the Asian group, MST could not be calculated and is denoted as NA. MST, median survival time; HR, hazard ratio; 95% CI, 95% confidence interval; BMI, body mass index; NA, not available.

Figure 1. mRNA expression levels of ATAD3 genes in hepatocellular carcinoma and normal liver tissues in The Cancer Genome Atlas database. Red bars denote the tumor tissue results, while grey bars denote the normal tissue results. *P<0.05 compared with normal liver tissues. ATAD3, ATPase family AAA domain-containing protein 3; T, tumor; N, normal; LIHC, liver hepatocellular carcinoma; TPM, Transcripts Per Million.
LIU et al.: ATAD3A AND ATAD3B EXPRESSION ARE SIGNIFICANTLY ASSOCIATED WITH OVERALL SURVIVAL OF HCC

ATAD3A and ATAD3B expression are significantly associated with overall survival of HCC. (Table II; Fig. 2D).

### Table II. Prognostic survival analysis of ATAD3 gene expression in The Cancer Genome Atlas database.

| Gene       | Patients (n=360) | No. of events (%) | MST (days) | HR (95% CI)     | P-value | Adjusted HR (95% CI) | Adjusted P-value |
|------------|-----------------|------------------|------------|-----------------|---------|---------------------|-----------------|
| ATAD3A     |                 |                  |            |                 |         |                     |                 |
| Low        | 180             | 52 (28.9)        | 2,456      | 1.54 (1.08-2.20) | 0.017   | 1.52 (1.04-2.22)    | 0.032           |
| High       | 180             | 74 (41.1)        | 1,386      |                 |         |                     |                 |
| ATAD3B     |                 |                  |            |                 |         |                     |                 |
| Low        | 180             | 55 (30.6)        | 2,131      | 1.49 (1.05-2.13) | 0.026   | 1.52 (1.04-2.21)    | 0.031           |
| High       | 180             | 71 (39.4)        | 1,386      |                 |         |                     |                 |
| ATAD3C     |                 |                  |            |                 |         |                     |                 |
| Low        | 180             | 61 (33.9)        | 1,791      | 1.00 (0.70-1.42) | 0.993   | 0.88 (0.61-1.27)    | 0.490           |
| High       | 180             | 65 (36.1)        | 1,685      |                 |         |                     |                 |

*Adjusted for sex, age, tumor stage. MST, median survival time; ATAD3, ATPase family AAA domain-containing protein 3; HR, hazard ratio; 95% CI, 95% confidence interval.

Figure 2. Survival curves for ATAD3 gene expression in TCGA database. Survival curves of (A) ATAD3A, (B) ATAD3B and (C) ATAD3C. (D) Survival curves for joint-effects analysis of the combinations of ATAD3A and ATAD3B in TCGA database. TCGA, The Cancer Genome Atlas; ATAD3, ATPase family AAA domain-containing protein 3.

GO functional analysis of ATAD3 genes. KEGG pathway analysis revealed that the ATAD3 gene cluster was associated with ATP binding, cell growth and cell division. Particularly, ATAD3A was a possible negative regulator of apoptosis (Table IV). Biological networks constructed by Cytoscape indicated that ATAD3 genes serve important roles in ATP binding, nucleoside binding, nucleotide binding, purine nucleotide and ribonucleotide binding, adenylyl cyclase activity, hydrolase activity, pyrophosphatase activity and nucleoside-triphosphatase activity (Fig. 3).
ATAD3, a member of the ATPase family, is exclusively present in multicellular eukaryotes at the interface between the outer and inner mitochondrial membranes, where it controls mitochondrial dynamics, mitochondrial fission, proliferation, and cholesterol transport (16,29,30). In particular, one cellular function of ATAD3 is protecting mtDNA integrity in multicellular organisms (14,16). A number of studies have demonstrated that ATAD3 is linked to the progression of various malignancies, including non-Hodgkin’s lymphoma (21), lung adenocarcinoma (22), uterine cervical cancer (23) and prostate cancer (24). However, to the best of our knowledge, there have been no previous reports that have identified the association of ATAD3 with HCC. Therefore, this is the first study to indicate an association between ATAD3 expression and HCC outcomes.

In the present study, the expression of all ATAD3 genes, including ATAD3A, ATAD3B and ATAD3C, was analyzed with regard to the prognosis of patients with HCC from TCGA database. The results indicated that ATAD3A and ATAD3B expression were significantly associated with OS in HCC. High ATAD3A expression or high ATAD3B expression were associated with poor MST and OS in patients with HCC. In addition, a joint-effects analysis demonstrated that patients with high ATAD3A and ATAD3B expression had reduced MST and OS rates. However, the mechanism underlying the poor survival of patients with HCC with high ATAD3A and ATAD3B expression requires further investigation.

ATAD3A is the human homologue of murine TOB3 (20), which controls mitochondrial dynamics at the interface of the inner and outer mitochondrial membranes and regulates diverse cellular responses including growth, cholesterol channeling and mitochondrial fission (16). ATAD3A has been reported to be associated with various malignancies.
to indirectly interact with mtDNA, and silencing of ATAD3A increases the condensation and decreases the multimerization of mtDNA (14). A report indicated that ATAD3A was overexpressed in lung adenocarcinoma samples and associated with significantly higher tumor recurrence and increased drug resistance, and that silencing ATAD3A increased apoptosis in lung adenocarcinoma cells (22). Another study suggested that ATAD3A was highly expressed in prostate cancer, and that downregulating ATAD3A expression reduced prostate-specific antigen secretion and cisplatin resistance (24). ATAD3A is also associated with HPV infection, reduced autophagy and apoptosis, and increased drug resistance in uterine cervical cancer (23). Additionally, ATAD3B, which is a c-MYC and myogenin target gene, was reported to serve important roles in tumor progression (8).

Regarding ATAD3B, a study demonstrated that this family member was downregulated in radiation-treated Raji B cells and was associated with proliferation and apoptosis inhibition (21). Another study reported that higher ATAD3B expression was associated with poor survival in breast cancer and that ATAD3B was activated through estrogen receptor-α-mediated, non-genomic, MAPK-regulated transcription factors, including myogenin and c-Myc (31). Notably, one study suggested that ATAD3B overexpression results in loss-of-function of endogenous ATAD3A (32). This was corroborated by a study that indicated ATAD3B, as a human embryonic stem cell-specific mitochondrial protein, negatively regulated ATAD3A and acted as an adaptor of mitochondrial homeostasis and metabolism in human embryonic stem cells and lung carcinoma cells (33). In the present study, gene function network analysis also indicated that ATAD3A negatively regulated apoptotic processes. ATAD3C has been reported to have 87% homology with ATAD3A and is also associated with tumor progression (11).

However, to the best of our knowledge, no studies have reported that ATAD3C expression levels are associated with the prognosis of human patients with cancer. In the current study, ATAD3C was not significantly associated with patient survival with HCC.

In conclusion, previous studies have reported that ATAD3A and ATAD3B are associated with tumor progression, likely due to their roles in proliferation, apoptosis, autophagy and increasing drug resistance (11,22,23). The present study's gene network analysis also revealed that the ATAD3 protein family was associated with cell growth, cell division and apoptosis. Results also demonstrated that ATAD3A and ATAD3B expression levels were significantly associated with the prognosis of patients with HCC. The present study revealed that ATAD3A and ATAD3B may serve as potential biomarkers for predicting the prognosis of patients with HCC. However, experimental and multi-center studies of ATAD3 are required to further confirm the present study's results.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

BY, XL and GL designed the study. LA, QY and TY analyzed the data and interpreted the results. XL and GL wrote the manuscript. BY edited the manuscript. All authors discussed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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