Overexpression of SLC25A15 is involved in the proliferation of cutaneous melanoma and leads to poor prognosis

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Introduction

Melanoma is a skin tumor with a high degree of malignancy, poor prognosis and few effective therapies. Deprivation of the arginine from cancer cells through transport inhibition and arginine depletion is a novel strategy for cancer therapy. In this study, we have investigated the effect of SLC25A15, which encodes the mitochondrial ornithine carrier 1, on melanoma progression. Using bioinformatics methods to screen the data from TCGA and GEO, we found that SLC25A15 is overexpressed in patients with melanoma and negatively related with the overall and disease-free survival rates. Knockdown the expression of SLC25A15 by siRNA could effectively inhibit the proliferation of A375 melanoma cells, as detected by CCK8 and colony formation. Furthermore, SLC25A15 siRNA was able to promote apoptosis of A375 cells, which exhibited decreased expression levels of the anti-apoptotic protein Bcl-2 while showing increased pro-apoptotic protein Bax and cleaved caspase-3. All these results suggest that the overexpression of SLC25A15 is involved in the progression of melanoma and may predict the prognosis of melanoma. This may shed new lights on the diagnosis and therapy of melanoma in the future. <

Key words: A375 cells, apoptosis, cutaneous melanoma, proliferation, SLC25A15.
Cell culture
The cell lines used throughout this study included human cutaneous melanoma (A375, MV3) and normal human primary epidermal melanocytes (HEMa), which were all purchased at the ATCC (USA). Cells were routinely cultured in a Dulbecco's Modified Eagle's Medium (DMEM) (ATCC® 30-2002™, USA) at 37 ºC in a 5 % CO2 humid atmosphere, supplemented with 100 U/mL penicillin, 0.1mg/mL streptomycin and 10% heat-inactivated fetal calf serum. Once the adherent cells reached the logarithmic phase of growth, a trypsin solution was added onto the cell layer to get single cell suspensions for further experiments.

Cell transfection
When cell density in the culture plate reached about 80%, they were transfected with small interfering SLC25A15 RNA (si-SLC25A15, F: 5'-GUGGAAUACGAAUCAAGC-3'), or non-specific control si-RNA (si-con) using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. After a 48 h culture, the interference effect of si-RNA was evaluated by quantitative real-time reverse transcription-PCR (qRT-PCR) and the cells were used in the subsequent experiments.

Materials and Methods

Analysis of TCGA and Oncomine Data
To determine the expression pattern of SLC25A15 in melanoma, the datasets in The Cancer Genome Atlas (TCGA) and Oncomine database (https://www.oncomine.org) were used. Briefly, we used GEPIA (Gene Expression Profiling Interactive Analysis), a web-based tool to perform the differential gene analysis (Tumor: n=461; Normal: n=558) and survival analysis based on Skin Cutaneous Melanoma (SKCM) data in TCGA [20]. Moreover, SLC25A15 gene was queried in Oncomine and the results were filtered by selecting melanoma vs. Normal Analysis.

Figure 1. Overexpression of SLC25A15 in melanoma patients from TCGA and Oncomine databases. A. Data from TCGA database. Tumor group: red column; non-tumor group: gray column. B-C: data from Oncomine database (Riker [21] and Talantov [22]) are plotted. The boxes represent data from the 25th to 75th percentiles. The horizontal lines are the medians. The whiskers represent the 10th and 90th percentiles, respectively.
microscope in 5 random view fields. This experiment was repeated in triplicate.

Western blot analysis
Cells were harvested 48-h post-transfection. Total proteins were extracted by Radio Immunoprecipitation Assay (RIPA) Buffer (Beyotime Inc., Shanghai, China). The protein concentrations were then quantified by the BCA Protein determination method (Beyotime Inc., Shanghai, China). Proteins (20µg / lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane. After blocking non-specific binding sites with 5% non-fat dried milk for 1 h, the membranes were incubated with primary antibodies (ORC1, 1:1000, Invitrogen; Bcl-2, 1:1000, caspase-3, 1:1000; β-actin, 1:5000, all from Proteintech) overnight at 4˚C. The membranes were then incubated with HRP-conjugated secondary antibody at room temperature for 1 h. Finally, the protein bands were visualized using ECL reagents (Pierce). β-actin was used as a house-keeping protein and the relative expression was calculated as a target protein / β-actin ratio. Colored bands were scanned and analyzed by QUANTITY ONE software.

Statistical analysis
Kaplan-Meier method and Log-rank test were used for survival analysis. The comparison of two groups was

Figure 2. Poor prognosis of SLC25A15 overexpression in melanoma patients from TCGA database. A. SLC25A15 high expression is associated with a poor overall survival in melanoma patients. B. SLC25A15 high expression is associated with a poor disease-free survival in melanoma patients. Kaplan–Meier plots of survival were generated by the software from Gepia (http://gepia.cancer-pku.cn) using the data from TCGA.
performed by Student’s t-test. When more than two groups were compared, the One-way ANOVA analysis was used. All statistical analyses were performed using the SPSS 22.0 (SPSS Inc., Chicago, IL, USA) software. The data were presented as mean ± standard deviation (SD).

Results

Up-regulation of SLC25A15 expression in melanoma
First, we analyzed the SLC25A15 mRNA expression between melanoma and normal skin tissues using the publicly available database TCGA and Oncomine. From the data of TCGA, we found that the expression of SLC25A15 was up-regulated significantly in the melanoma group as compared with the normal group (P < 0.05, Figure 1A). Moreover, we performed an expression analysis of SLC25A15 using two datasets from Riker [21] and Talantov [22] melanoma cohorts from Oncomine. In both cohorts, the expression of SLC25A15 mRNA was significantly increased in melanoma tissues as compared to normal skin tissues (Figure 1B-C). Second, the relationship of SLC25A15 overexpression with prognosis in all 456 melanoma patients was also analyzed. Based on the median value of SLC25A15 expression, patients were divided into two groups with high (n = 229) and low (n = 227) SLC25A15 levels, respectively. The results showed that patients with high SLC25A15 mRNA expression had shorter overall and disease-free survival times than those with low SLC25A15 (both P < 0.01, Figure 2A-B). These results suggested that overexpressed SLC25A15 is correlated with a poor prognosis in melanoma patients.

SLC25A15 is overexpressed in cutaneous melanoma cells
To further characterize the expression of SLC25A15 in melanoma, we analyzed its mRNA level in normal melanocytes and some cutaneous melanoma cell lines, including A375, MV3, WM-115 and SK-MEL-3, using qRT-PCR. As shown in Figure 3, the expression of SLC25A15 in cells from the melanoma cell lines was higher than in the HEMa normal melanocytes (all P < 0.01). These results obtained in melanoma cell lines were consistent with the conclusion draw from database analyses. Moreover, the expression of SLC25A15 in A375 cells was the highest among all tested melanoma cell lines. Therefore, in the next in vitro experiments, A375 cells were used.

Decreased proliferation of SLC25A15-knocked down A375 cells
For better evaluating the effect of SLC25A15 on melanoma progression, a SLC25A15 siRNA (si-SLC25A15) was transfected into A375 cells. Forty-eight hours later, the expression of SLC25A15 mRNA and protein in A375 cells was detected by qRT-PCR and western blot to assess the effect of si-SLC25A15. Our results showed that si-SLC25A15 could significantly reduce the RNA and protein expression levels of SLC25A15 in A375 cells, and the knockdown efficiencies were over 70% (Figure 4A-C, P < 0.01). Then we studied the effect of SLC25A15 on cutaneous melanoma cells by using the siRNA. Firstly, cell proliferation activity was examined using a CCK-8 assay. Silencing of SLC25A15 remarkably inhibited the viability of A375 cells as compared to the control at 72 h (P < 0.05) and 96 h (P < 0.01). Subsequently, a colony formation assay was also performed. We found that A375 cells transfected with SLC25A15 siRNA formed fewer clones than the control (Figure 4E-F, P < 0.01). These data suggest that SLC25A15 may play a promoting role in melanoma proliferation.

Decreased expression of SLC25A15 promotes the apoptosis of A375 cells
Given the fact that the apoptosis plays an important role in controlling cell proliferation, we then tested the expression of apoptosis-related proteins in A375 cells with or without siRNA treatment. The western blot results showed that the expression levels of the anti-apoptotic protein Bcl-2 was decreased; by contrast, the pro-apoptotic protein Bax and cleaved caspase-3 levels were increased (Figure 5E-F, P < 0.01). These results indicate that SLC25A15 could inhibit the apoptosis of A375 cells.

Discussion
To our knowledge, there is no study showing the effect of SLC25A15 in melanoma. In this study, for the first time, we identified an overexpression of SLC25A15 in melanoma, which was negatively related with the survival rate of patients. Moreover, we also found an up-regulation of SLC25A15 in some melanoma cells.
might accelerate the endogenous synthesis of arginine to compensate for the arginine auxotrophy. Moreover, we also found the overexpression is negatively related with the survival rate of patients. These results suggest that \textit{SLC25A15} is a promising novel diagnostic marker and therapeutic target for human skin melanoma in the future.

Early findings suggest that exogenous arginine deprivation can induce apoptosis and cause some of the death of cells from cancer cell lines, including breast cancer, prostate cancer, mesothelioma, and melanoma cell lines [30-33]. Although the signaling pathway responsible for this apoptosis is still unknown, it is believed that the apoptosis caused by arginine elimination could be activated through caspase-dependent and/or independent pathways [11]. In the present study, we also showed that the content in the anti-apoptosis pro-

\begin{figure}[h]
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\caption{\textit{SLC25A15} knockdown inhibits the proliferation of A375 cells. A. Relative expression levels of \textit{SLC25A15} in A375 cells after treatment with small interfering RNA (q-PCR). B-C. Relative protein expression levels of ORC1 in A375 cells after treatment with small interfering RNA (Western blot). D. \textit{SLC25A15} knockdown decreases the viability of A375 cells as shown by a CCK8 assay. E-F. \textit{SLC25A15} knockdown inhibits colony formation of A375 cells in a colony formation assay. All values are presented as means ± S.D. * \textit{P}<0.05, ** \textit{P}<0.01 compared with si-con group. \textit{n}=5 for each group. si-con, scramble control siRNA.}
\end{figure}
tein Bcl-2 decreased, while those of the pro-apoptosis protein Bax and cleaved caspase-3 increased after SLC25A15 knockdown. Our findings are consistent with previous reports and suggest that the targeting of SLC25A15 may be a novel anti-melanoma method through promoting apoptosis.

In conclusion, our study firstly reported that the overexpression of SLC25A15 is an important biomarker of melanoma development. The inhibition of the SLC25A15 expression could effectively block the proliferation of A375 melanoma cells via promoting apoptosis. It might shed new lights on the diagnosis and therapy of cutaneous melanoma. However, our study is a preliminary study that needs more works to be done to clarify the roles and mechanisms of SLC25A15 in melanoma.

CONFLICT OF INTEREST
The authors state that there are no competing interests in this work.

REFERENCES


Figure 5. Silencing SLC25A15 promotes the apoptosis of A375 cells. (A) Representative images of the expression of Bcl-2, Bax and cleaved caspase-3 by western blot analysis. (B) Quantification of Bcl-2, Bax and caspase-3 protein expression. All values are presented as means ± S.D. **P<0.01 compared with si-con group. n=5 for each group. si-con, scramble control siRNA.
REFERENCES


