Comprehensive Analysis and Validation of Solute Carrier Family 25 (SLC25) in Pan-Cancer

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Primary research

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Abstract

Background

As the largest gene family functioning in protein transport among human solute carriers, the SLC25 family (mitochondrial carrier family) has been reported to be associated with the genesis and development of many diseases. However, a comprehensive exploration for the roles of SLC family in cancer remains lacking.

Method

In the present study, a total of 15 functional SLC25 family genes were retrieved from all current publications. And multi-dimensional analyses were systematically performed based on the transcriptome and genome data of SLC25 family from a variety of online databases for their expression, mutation and copy number variation, cancer prognosis, signaling pathways and immune cell infiltration. Validation by qPCR was further conducted for the expression of partial SLC25 family members in some tumor tissue.

Results

We found that SLC25A7 was highly expressed in stomach adenocarcinoma, kidney chromophobe and colon adenocarcinoma, while low expressed in lung adenocarcinoma and thyroid carcinoma as well as head and neck squamous cell carcinoma. SLC25A23 was decreased in colon adenocarcinoma and head and neck squamous cell carcinoma. SLC25A4 was down-regulated in stomach adenocarcinoma, colon adenocarcinoma, kidney renal clear cell carcinoma and head and neck squamous cell carcinoma. Validation results of stomach adenocarcinoma and colon adenocarcinoma were consistent with our bioinformatic prediction. The analysis in regard to cancer-related pathways indicated that SLC25A5 was more likely to be positively associated with carcinogenic pathways such as PI3K-AKT-MTOR, MYC-TARGETS-V2 and E2F-TARGETS. Immune cells including Macrophages M2 and B cells naïve had significant association with SLC25s expression. Survival analysis demonstrated that SLC25A8 was linked to a high-risk effect on the prognosis of cervical squamous cell carcinoma and adenocarcinoma significantly. The majority of SLC25 genes showed high mutation frequency in uterine corpus endometrial carcinoma with an overall average mutation rate of 0.100189. SLC25A8 had extensive copy number variation in different cancer tissue. And SLC25A25 showed high mutation frequency in breast cancer cell lines. As for the influence of mutation on gene expression and cancer prognosis, SLC25A4 mutation could alter its expression in uterine corpus endometrial carcinoma. Most SLC25 gene mutation made prominent effects on the prognosis of patients with uterine corpus endometrial carcinoma.

Conclusion

All these findings suggested that the SLC25 family might be crucial to the occurrence, progression and prognosis of tumor. They had the potential to be predictive biomarkers for early diagnosis and prognosis as well as novel targets for individualized treatment of cancer.

1. Introduction

As the center of energy metabolism, mitochondria play vital roles in human physiological activities, and substantial material exchange occurs in mitochondrial membrane at all times. Mitochondrial membrane consists of outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM). The permeability of OMM is relatively high, while IMM has a lower permeability with higher requirement for penetration. Only proteins with a molecular weight of less than 15KD can pass through IMM [1]. Therefore, some carriers on IMM are essential for transmembrane transport to participate in important cellular activities, such as fat metabolism, oxidative phosphorylation and biomacromolecule synthesis [2]. The mitochondrial carrier family (SLC25) is the largest gene family functioning in protein transport among human solute carriers, with 53 members discovered already. These carrier proteins transport macromolecular solutes across IMM regardless of molecular size limitation and contribute in many cellular processes.

In recent years, knowledge for the function of SLC25 family has been increasingly enriched with continuous in-depth research. Lots of experiments have confirmed that SLC25s exert regulatory roles in all aspects of physiological process, pathological process and disease progression[3, 4]. Numerous studies have indicated that SLC25 family is associated with cancer with the potential to be a novel tumor biomarker, which has profound significance for cancer diagnosis, treatment and prevention[5].

Currently, however, the function of many SLC25 genes has not been elucidated yet, and specific mechanism needs to be explored for the influence of SLC25 family on tumor biological behaviors. Here, multi-dimensional analyses were systematically performed for the expression status (tissue mRNA, protein and cell lines) of SLC25 family from the perspective of pan-cancer based on a variety of online databases. And their association with tumor-related pathways, immune cell infiltration and prognosis in pan-cancer was subsequently analyzed. In addition, the impacts of mutation and copy number variation on their expression in cancer were also investigated. Our study aims to provide theoretical basis for the potential of SLC25 family as tumor biomarkers and targets.

2. Materials And Methods

2.1 Data collection

2.1.1 Selection of SLC25 family

A total of 53 SLC25 family genes were selected from published review articles. And they were manually converted into Ensemble gene IDs and HGNC symbols according to the Gene Cards (<https://www.genecards.org/>).

2.1.2 Collection of TCGA data

Information about 33 different types of cancer was collected from the TCGA database (<http://cancergenome.nih.gov/>) (Table S1), including TPM (Transcripts Per Kilobase Million) expression, copy number variation and mutation as well as clinical information (survival status, stage, grade and survival time) downloaded from UCSC Xena (<https://xenabrowser.net/>).

2.1.2 Collection of proteomics data

The protein expression of SLC25 family was obtained from the Human Protein Atlas (<https://www.proteinatlas.org/>) containing 21 types of cancer and corresponding normal tissue. We selected 10 types of relatively common cancer and collected the expression data of SLC25 family.

2.1.3 Collection of genome-wide mutation data in pan-cancer cell lines

The Cancer Cell Line Encyclopedia (CCLE) database (<https://portals.broadinstitute.org/ccle>) was utilized to evaluate the expression differences, mutation and copy number variation frequency of SLC25 family in 431 cell lines of 6 cancer types [6].

2.2 Multidimensional analysis for the expression of SLC25 gene family in pan-cancer

2.2.1 Differential expression analysis of SLC25 family in pan-cancer

The mRNA expression data of SLC25 family genes in pan-cancer was processed with Deseq2 R package to identify differentially expressed genes. The criteria were set as corrected $P < 0.05$ and at least 4-fold expression change ($|\log Fc| \geq 2$).

The protein expression levels of SLC25 family in pan-cancer were assessed by the percentage of patients with high and medium protein expression in cancer tissue from the Human Protein Atlas and cancer immunohistochemistry map.

Differentially expressed genes in pan-cancer cell lines were identified based on CCLE. Kruskal-Wallis rank test was employed to compare the expression of SLC25 family genes in different cancer cell lines.

2.2.2 Signal transduction pathway analysis of SLC25 family genes in pan-cancer

The association between SLC25 family expression and tumor-related pathways was evaluated by Gene Set Variation Analysis (GSVA), which was a non-parametric method to estimate gene enrichment alteration with expression array samples. The Pearson correlation coefficient (PCC) between SLC25 family expression and pathway activity was calculated to assess the association of SLC25 family with the activation or inhibition of some certain pathways. Pathways with $|PCC| > 0.3$ and adjusted $P < 0.05$ were considered to be significantly associated with SLC25 genes.

2.2.3 Association analysis of SLC25 family expression with immune cell infiltration in pan-cancer

The association between SLC25 family and immune cell infiltration was analyzed by calculating the Spearman correlation coefficient (SCC). Records with $|\text{SCC}| > 0.3$ and adjusted $P < 0.05$ were determined to have statistical significance.

2.2.4 Association analysis of SLC25 family expression with clinical prognosis in pan-cancer

To investigate the association between SLC25 family genes and the survival of cancer patients, all cases were divided into two groups according to the median expression of SLC25 family. The inter-group survival rates were compared by log-rank test, and $P < 0.05$ was regarded as statistically significant.

2.3 Analysis for the mutation and copy number variation of SLC25 family in pan-cancer

2.3.1 Mutation analysis of SLC25 family in pan-cancer

Based on the mutation data obtained from TCGA and CCLE, the percentage of gene mutation was determined as the mutation frequency of SLC25 family in cancer tissue and cell lines.

2.3.2 Copy number variation analysis of SLC25 family in pan-cancer

The copy number variation of SLC25s in different cancer tissue and cell lines was identified based on the CNV data from TCGA and CCLE. Then the CNV frequency in each tumor tissue and cell line was estimated with the ratio of CNV amplification and deletion.

2.4 Correlation analysis of SLC25 family gene mutation and copy number variation with their expression in pan-cancer

The correlation of SLC25 family gene mutation and copy number variation with their expression was calculated with Mann-Whitney U test by R software.

2.5 Validation by quantitative PCR (qPCR) in vivo

We collected clinical specimens from the First Hospital of China Medical University to validate the expression differences of SLC25 family genes in pan-cancer at mRNA level, differentially expressed SLC25 genes were validated in several cancer by real-time PCR. This study was reviewed and approved by the ethics committee of the First Hospital of China Medical University. In total, 23 pairs of gastric cancer and adjacent tissues, as well as 30 pairs of colorectal cancer and adjacent tissues, were included

to detect the relative mRNA levels of SLC25s. Total RNA was extracted from samples using TRIzol. Relative quantification for SLC25A4, SLC25A7 and SLC25A23 in gastric cancer and colon cancer tissue was performed with SYBR Green kit (Takara, Japan) and real-time PCR system. The information of primer sequences was listed in Table S2. qPCR results were standardized with the $2^{-\Delta\Delta CT}$ method quantified by β -actin. And the specificity of PCR products was confirmed by melting curve analysis. The expression of SLC25 genes in tumor tissue was evaluated with rank sum test in SPSS software, and $P < 0.05$ was considered statistically significant.

3. Results

3.1 Expression profiles of SLC25 family in pan-cancer

3.1.1 Expression of SLC25 family at tissue mRNA level

All 53 genes of SLC25 family were retrieved from published reviews for the detection of expression level. Due to the unknown function of some genes, we selected 15 genes of SLC25s with accurate function for the study. Foremost, the expression differences of SLC25s in various tumor tissue were analyzed based on TCGA data (Fig. 1). The results showed that SLC25 family was differentially expressed in 33 types of cancer. In stomach adenocarcinoma, SLC25A7 had increased expression while SLC25A4 was decreased. Only SLC25A25 was low expressed in bladder urothelial carcinoma. SLC25A7 was highly expressed in kidney chromophobe. SLC25A24 was up-regulated in hepatocellular carcinoma while SLC25A25 was down-regulated. In colon cancer, SLC25A7 expression was elevated while SLC25A23 was reduced. SLC25A31 was down-regulated in glioblastoma multiform. In kidney renal clear cell carcinoma, all the expression of SLC25A31, SLC25A5, SLC25A4 and SLC25A25 was significantly decreased. In lung adenocarcinoma, SLC25A7 and SLC25A25 had decreased expression while SLC25A41 was elevated. SLC25A7 and SLC25A25 were reduced in uterine Corpus Endometrial Carcinoma. In head and neck squamous cell carcinoma, SLC25A9, SLC25A23, SLC25A4 and SLC25A5 all showed decreased expression. In lung squamous cell carcinoma, SLC25A27 and SLC25A25 were reduced. In thyroid cancer, significant down-regulation was observed in SLC25A7 and SLC25A25. Besides, SLC25A25 was not only reduced in kidney renal papillary cell carcinoma but also breast invasive carcinoma. Moreover, SLC25A7 and SLC25A27 had decreased expression in breast invasive cancer while SLC25A41 was increased. Additionally, SLC25A25 was significantly down-regulated in 10 of 33 types of cancer tissue (Fig. 1A). The expression of SLC25A7 in each cancer was shown in Fig. 1B.

3.1.2 Expression of SLC25 family at tissue protein level

According to the immunohistochemical data from Human Protein Atlas, SLC25 family had differential protein expression in different cancer tissue. The protein expression of SLC25A4, SLC25A5 and SLC25A6 was consistent in top 10 common cancer types. They were highly expressed in lung squamous cell carcinoma, colon adenocarcinoma, liver hepatocellular carcinoma, breast invasive carcinoma, cervical squamous cell carcinoma, endocervical adenocarcinoma and uterine corpus endometrial carcinoma.

SLC25A8 and SLC25A12 also had high expression levels in some cancer such as colon adenocarcinoma, bladder urothelial carcinoma, prostate adenocarcinoma, breast invasive cancer and uterine corpus endometrial carcinoma. On the contrary, SLC25A23 and SLC25A13 had medium-to-low protein expression levels in some cancer. The expression of SLC25A9 and SLC25A31 was low or undetected. SLC25A24 was highly expressed in the 10 common cancer types (Fig. 2A). The immunohistochemical data of SLC25A24 in different cancer tissue from the Human Protein Atlas showed that SLC25A24 had higher expression in 16 types of cancer including breast invasive cancer, colon adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, stomach adenocarcinoma, thyroid carcinoma and uterine corpus endometrial carcinoma (Fig. 2B).

3.1.3 Expression of SLC25 family in cell lines

The analysis based on CCLE data suggested that some members of SLC25 family were highly expressed in breast, colorectal, stomach, liver, lung and ovarian cancer cells, including SLC25A5, SLC25A6 and SLC25A8 (Fig. 3A). SLC25A5 had high expression level in both male and female cases of cancer cells mentioned above. SLC25A6 was also increased in most cancer patients. SLC25A23 was mainly expressed in lung cancer and ovarian cancer cells to some extent. The expression level of SLC25A5 in different tumor cell lines from CCLE was shown in Fig. 3B.

3.2 Association of SLC25 family genes with signaling pathways in pan-cancer

Next, we analyzed and visualized the association of SLC25 family with signaling pathways in pan-cancer ($P < 0.05$, $|R| > 0.3$), and the correlation coefficient was calculated to explore the potential mechanism of SLC25 family in tumorigenesis (Fig. 4A). SLC25 family was shown to be associated with the activation or inhibition of various tumor-related pathways. SLC25A5 and SLC25A9 were more likely to be involved in carcinogenic pathways, mainly including PI3K_AKT_MTOR pathway, MYC_TARGETS_V1 pathway, MYC_TARGETS_V2 and MTORC1 pathway. Moreover, SLC25A23 and SLC25A4 were also related to these pathways. The number of tumor-related pathways associated with each gene of SLC25 and their influence on pathway activity were presented in Fig. 4B.

Furthermore, it was found that multiple genes of SLC25 could be involved in a same pathway with consistent correlation. For example, both SLC25A4 and SLC25A23 were negatively correlated with the E2F-TARGET and MYC-TARGET-V2 pathways. Besides, SLC25A12 and SLC25A14 were negatively correlated with the XENOBIOTIC-METABOLISM pathway. We speculated that the two genes involved in the same pathway might have synergistic effects or exert upstream and downstream regulatory roles in each other. Therefore, the correlation between these genes was further excavated suggesting that SLC25A4/SLC25A23 and SLC25A14/SLC25A12 might synergistically function in the E2F-TARGET, MYC-TARGET-V2 and XENOBIOTIC-METABOLISM pathways, respectively (Fig. 4C).

3.3 Association of SLC25 family with tumor immune cell infiltration

Then the association of SLC25 family with immune cell infiltration in different cancer was investigated. The maximal correlation between those genes and each type of immune cell infiltration was shown in Fig. 4D. SLC25A6 was most correlated with Macrophage M1 in uveal melanoma. Generally, SLC25 family had strong correlation with the following immune cells including Macrophages M2, T cell CD8, T cell CD4 memory activated and T cell CD4 memory resting (Fig. 4E).

3.4 Association of SLC25 family expression with prognosis in pan-cancer

The prognostic significance of SLC25 family was evaluated by Cox regression analysis. The effects varied with SLC25 family genes and cancer types (Fig. 5A). SLC25 family was found to be significantly associated with the overall survival rate in at least one of 33 cancer types. Some genes of SLC25 were associated with poor prognosis of patients in cervical squamous cell carcinoma and endocervical adenocarcinoma, including SLC25A4, SLC25A5, SLC25A8, SLC25A14, SLC25A12, SLC25A23 and SLC25A41. In contrast, the expression of SLC25A4, SLC25A8, SLC25A12 and SLC25A25 was associated with better prognosis of patients in acute myeloid leukemia. Some other SLC25s made contrary effects on the prognosis of different cancer. For example, the SLC25A8 gene could lead to poor prognosis of patients in bladder urothelial carcinoma, skin cutaneous melanoma, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma and thymic carcinoma. However, it might prolong the survival in acute myeloid leukemia, kidney renal clear cell carcinoma, uveal melanoma and brain low-grade glioma. As shown in Fig. 5B, SLC25A8 had significantly increased risk effects on the prognosis of cervical squamous cell carcinoma and adenocarcinoma.

3.5 Genetic variation of SLC25 family in pan-cancer

Next, the mutation frequency of SLC25 family was investigated based on TCGA data. Almost all SLC25s had high mutation frequency in uterine corpus endometrial carcinoma, including SLC25A12, SLC25A13, SLC25A14, SLC25A23, SLC25A24 and SLC25A25. However, several genes such as SLC25A4 and SLC25A5 showed low mutation frequency in most cancer with an overall average mutation rate of 0.100189 (Fig. 6A). Regarding the copy number variation (CNV) of SLC25 family, SLC25A8 demonstrated extensive CNV in different cancer types (Fig. 6B). SLC25A4 and SLC25A24 had more copy number deletion in cholangiocarcinoma, sarcoma and lung squamous cell carcinoma. In addition, the mutation of SLC25s was detected in different cancer cell lines based on CCLE data (Fig. 6C). It was found that the mutation rate of SLC25A25 was highest in breast cancer cell lines and SLC25A24 had a higher mutation level in colorectal cancer cell lines. Relatively high mutation rates were also observed in ovarian cancer cell lines for SLC25A13, SLC25A24, SLC25A41 and SLC25A23; and in skin cancer cell lines for SLC25A13, SLC25A8 and SLC25A12.

3.6 Effects of the mutation in SLC25 family on expression and cancer prognosis

Then we explored whether the mutation in SLC25 family could influence their expression and cancer prognosis (Fig. 7A). The mutation of SLC25A13 in colon cancer was suggested to have the most significant effect on its expression. In uterine corpus endometrial carcinoma, the mutation of SLC25A4, SLC25A25 and SLC25A27 all had certain impacts on their expression. SLC25A23 mutation was significantly correlated with its expression in gastric cancer. In skin melanoma, the mutation of SLC25A24 and SLC25A27 could affect their expression. SLC25A6 mutation made significant effect on its expression in ovarian serous cystadenocarcinoma. SLC25A24 mutation could alter its expression in skin melanoma, hepatocellular carcinoma and colon cancer. In colon cancer, both the expression of SLC25A13 and SLC25A9 could be affected by their mutation. All above-mentioned results reached statistical significance ($P < 0.05$).

Furthermore, association analysis was performed for the mutation of SLC25 family and cancer prognosis by calculating the hazard ratio (Fig. 7B). SLC25A24 mutation showed the highest HR in breast invasive cancer among all SLC25 genes, and it also showed certain risk effects in mesothelioma, ovarian serous cystadenocarcinoma and thyroid carcinoma. SLC25A25 mutation was associated with poor prognosis in cervical squamous cell carcinoma and adenocarcinoma, cholangiocarcinoma and ovarian serous cystadenocarcinoma. Besides, the mutation of many SLC25s made high-risk effects on the prognosis of ovarian serous cystadenocarcinoma such as SLC25A8, SLC25A24, SLC25A25 and SLC25A31.

3.7 Effects of the copy number variation in SLC25 family on expression

Other than gene mutation, we also studied the influence of copy number variation in SLC25 family on gene expression in cancer. All the CNV of SLC25s was shown to affect their expression to some degrees. Among them, the CNV of SLC25A4 had statistical significance in most cancer, which was positively correlated with SLC25A4 expression (Table 1).

3.8 Validation of SLC25 family expression in vivo by qPCR

To validate the analytic results mentioned above, several differentially expressed genes were selected for qPCR validation, including SLC25A4 in gastric cancer, SLC25A23 in colon cancer and SLC25A7 in gastric & colon cancer. It was found that SLC25A4 was significantly down-regulated in gastric cancer (Fig. 8A). SLC25A23 had significantly low expression in colon cancer (Fig. 8B). The expression of SLC25A7 was increased in STAD (Fig. 8C), which was consistent with our bioinformatic prediction. However, the alteration of SLC25A7 expression in COAD did not reach statistical significance (Fig. 8D).

4. Discussion

In the present study, we explored the roles of SLC25 family in the genesis and development of cancer. According to current publications, a total of 15 SLC25 family genes were screened out. Multi-dimensional analyses were systematically performed based on the transcriptome and genome data of SLC25 family from a variety of online databases for the association among their expression, mutation and copy

number variation, cancer prognosis, signaling pathways and immune cell infiltration. Validation by qPCR was further conducted for the expression status of partial SLC25 family members in some tumor tissue.

Our study demonstrated that SLC25 family had differential expression in both tissue mRNA and protein as well as cell lines. At tissue mRNA level, SLC25A5, SLC25A27, SLC25A23, SLC25A4 and SLC25A25 were down-regulated in some cancer including kidney chromophobe, head and neck squamous cell carcinoma, breast invasive carcinoma and lung adenocarcinoma. At tissue protein level, the majority of SLC25 family genes had medium to high expression levels in 10 common cancer types such as lung adenocarcinoma, colorectal cancer, breast cancer and other tissue. For detailed results at tissue mRNA level, SLC25A4 was low expressed in stomach adenocarcinoma, head and neck squamous cell carcinoma, colon adenocarcinoma and kidney renal clear cell carcinoma. SLC25A7 was highly expressed in stomach adenocarcinoma, kidney chromophobe and colon adenocarcinoma but low in lung adenocarcinoma, thyroid cancer and breast cancer. SLC25A23 had low expression in colon cancer as well as head and neck squamous cell carcinoma. Therefore, SLC25A4, SLC25A7 and SLC25A23 were selected for subsequent validation of in situ expression in fresh cancer tissue by qPCR. We found that SLC25A4 and SLC25A23 were respectively decreased in gastric cancer and colon cancer, while SLC25A7 was increased in gastric cancer, which was consistent with our bioinformatic prediction. It has been shown that the ANT1 protein encoded by SLC25A4 was low expressed in rhabdomyosarcoma [7]. Combining with this, SLC25A4 is located in mitochondria and involved in metabolic process by regulating ATP/ADP. Thus the decreased expression of SLC25A4 might lead to aberrant metabolism, participating in tumor progression. As a well-known uncoupling protein, SLC25A7 also functions in important metabolic process in mitochondria, while its effect in tumor has been rarely studied. Similar findings could be observed in previous research for the expression of SLC25A7 in tumor. Alexandra et al. reported that SLC25A7 was highly expressed in squamous cell carcinoma of non-small cell lung cancer and closely related to glycolysis [8]. SLC25A23 is a Ca^{2+} sensitive mitochondrial carrier with the ability to effectively transport ATP, ADP and AMP except for Ca^{2+} . Its expression in cancer might be associated with abnormal ATP levels. The study would provide theoretical basis for their application as potential targets of cancer therapy including SLC25A4, SLC25A7 and SLC25A23.

The association analysis of SLC25s expression with tumor-related pathways revealed 35 pathways associated with SLC25s such as proliferation-related pathways, Ras and MYC pathways. The expression of SLC25 genes varied with each pathway, suggesting that different SLC25 genes might exert diverse roles in tumor. Among them, SLC25A5 was more likely to be involved in carcinogenic pathways. It has been reported that the SLC25A5 gene is up-regulated in most tumor[9–12], mainly encoding the ANT2 protein specifically expressed in proliferative tissue[13]. Several pathways shown to be positively associated with SLC25A5 were common tumor-related pathways regulating proliferation, including PI3K_AKT_MTOR, MYC_TARGETS_V2 and E2F_TARGETS[14–17]. ANT inhibitors have been considered as potential therapeutic targets for cancer [18]. Therefore, SLC25A5 might participate in cancer initiation and development via these oncogenic pathways. To elucidate the association hidden in SLC25 family genes differentially expressed in tumor could not only benefit the identification of novel molecules

involved in the regulation of tumor progression and better insights of pathogenesis, but also provide clues for candidate biomarkers used for cancer diagnosis, prognosis and treatment.

Additionally, immune cell infiltration also contributed to the effects of SLC25 family in tumor. SLC25 family was previously reported to be relevant with inflammation[19]. Kai Yasukawa et al. found that SLC25A12 was associated with the innate immunity of body through mitochondrial metabolism[20]. Marie-Clotilde et al. showed that SLC25A8 was rapidly activated and mobilized during the anesthesia of immune cells [21]. Hence, the SLC25 family was related to immunity more or less. In our research, the association between SLC25s and immune cell infiltration was mainly manifested in Macrophage M2. The role of macrophages in tumorigenesis and development has already been confirmed. They had a two-way interaction with cancer cells, especially M2 polarization. Cancer cells induced M2 polarity to promote a series of cancer deterioration[22]. Various metabolites in tumor microenvironment promoted the M2 polarization of macrophages to accelerate tumor progression [23]. These may provide corresponding thoughts for the diagnosis and treatment of cancer with respect to SLC25 family and tumor microenvironment.

Concerning the association of SLC25s expression with cancer prognosis, SLC25A8 was associated with poor prognosis in cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma and thyroid cancer, while better prognosis in membranous melanoma and brain low-grade glioma. SLC25A8 was involved in many diseases such as cancer and cardiovascular diseases, etc. [24], and its expression was connected with chemoresistance[25]. Masaru Kawanishi et al. found that in ovarian serous carcinoma, patients with high expression of SLC25A8 were less sensitive to platinum-based chemotherapy and developed drug resistance[26]. A latest study by Wang et al. demonstrated that knocking down SLC25A8 in cervical cancer cell lines could delay or reduce the proliferation, migration and invasion of cervical cancer cells via Ras/MAPK/ERK signaling pathways. Thus it could be applied to predicting the prognosis of cervical cancer[27], which was consistent with our findings. Similarly, a research for breast cancer indicated that SLC25A8 overexpression might directly affect the mitochondrial membrane potential of tumor cells, inhibit cell apoptosis and promote tumor metastasis through the TGF- β pathway, resulting in the poor prognosis [28]. Kuai et al. also believed that SLC25A8 was highly expressed in human colon cancer tissue associated with the metastasis and poor prognosis of colon cancer[29]. Furthermore, we found that SLC25A8 made discrepant effects on the prognosis of different cancer, with the potential to be a versatile biomarker for cancer prognosis. Above all, the expression of SLC25 family showed diverse roles in cancer prognosis, possibly owing to the various substrates transported by SLC25 genes affecting cancer genesis and development.

Currently, numerous studies have proved that the occurrence and progression of tumor were closely related to genetic variation including mutation and copy number variation. The mutation and copy number variation of SLC25 family were also investigated based on TCGA data. It was shown that almost all SLC25 genes had high mutation frequency in uterine corpus endometrial carcinoma, which was a well-accepted tumor with extensive gene mutation [30]. Notably, SLC25A4 had more deletion of copy number in cholangiocarcinoma, sarcoma and lung squamous cell carcinoma. For its reason, the expression of

ANT1 protein encoded by SLC25A4 might reduce ATP transport and promote Rax release, easily leading to cell apoptosis and tumorigenesis[31–33]. Moreover, the mutation analysis of SLC25 family in tumor cell lines revealed that the mutation rate of SLC25A25 was highest in breast cancer, suggesting that SLC25A25 was more apt to mutate in the environment of breast tissue. The SLC25A25 gene could control the dynamic balance of ATP, and the missing of SLC25A25 may reduce the metabolic efficiency in mice[34]. We also analyzed the influence of mutation and CNV in SLC25 genes on their expression and cancer prognosis. SLC25A4 and SLC25A6 mutation mainly affect their expression in uterine corpus endometrial carcinoma and ovarian serous cystadenocarcinoma, respectively. It has been reported that cells pretreated with ANT inhibitors can attenuate the release of Cytc connected with mitochondrial bioenergy. Therefore, ANT mutations are involved in many human diseases [35], which is consistent with our research. As for the impacts of mutation on cancer prognosis, SLC25A24 mutation was quite probably associated with a high risk in the prognosis of BRCA. Besides, SLC25A24 could protect tumor cells from death. Thus it might promote tumor growth and metastasis causing poor prognosis when mutated[36]. Taken overall, genetic variation may be the key to the alteration of gene expression and cancer prognosis. The variation in SLC25 genes might play critical roles in the regulation of their expression and cancer prognosis. However, the specific mechanism needs to be further explored.

5. Conclusion

In summary, multi-dimensional analyses were systematically performed for the differential expression of SLC25 genes in 33 types of human cancer. Several genes were selected for the validation of expression in fresh cancer tissue by qPCR including SLC25A4, SLC25A7, and SLC25A23. The results showed that SLC25A4 and SLC25A23 were respectively decreased in gastric cancer and colon cancer, while SLC25A7 was increased in gastric cancer, which was consistent with our bioinformatic prediction. It may provide theoretical basis for their application as novel targets of cancer therapy. SLC25A5 might be involved in some carcinogenic pathways regulated by genetic variation, participating in cancer initiation and development. In addition, we also investigated the association of SLC25s with clinical prognosis, and the influence of genetic variation on their expression and cancer prognosis. All these findings suggested that the SLC25 family might be crucial to the occurrence, progression and prognosis of tumor. They could be considered as predictive biomarkers for early diagnosis and prognosis as well as potential targets for individualized treatment of cancer.

6. Abbreviations

SLC25: solute carrier family 25;qPCR: Quantitative Real-time PCR; OMM: outer mitochondrial membrane; IMM: inner mitochondrial membrane; HGNC: HUGO Gene Nomenclature Committee; TCGA: The Cancer Genome Atlas; TPM: Transcripts Per Kilobase Million; CCLE: Cancer Cell Line Encyclopedia; GSVA: Gene Set Variation Analysis; PCC: Pearson Correlation Coefficient; SCC: Spearman Correlation Coefficient; CNV: Copy Number Variation.

7. Declarations

Acknowledgements

Not applicable.

Authors' contributions

YY and QX conceived and designed this study. SS, AW, YL and AL collected and analyzed the data. AL, YL, LY carried out experiments. HD participated in the experiment. AL wrote the paper. QX, ZL, and YY revised the manuscript. All authors have agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are openly available in The Cancer Genome Atlas (TCGA) data portal (<https://tcga-data.nci.nih.gov/tcga/>), Human Protein Atlas (<https://www.proteinatlas.org/>), GeneCards (<https://www.genecards.org/>), UCSC Xena (<https://xenabrowser.net/>) and Cancer Cell Line Encyclopedia (CCLE) database (<https://portals.broadinstitute.org/ccle>). The rest of the data are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

A total of 23 pairs of gastric cancer and adjacent tissues, as well as 30 pairs of colorectal cancer and adjacent tissues were collected from patients who had undergone surgery at the First Hospital of China Medical University. The study was approved by the Ethics Committee of China Medical University (Shenyang, Liaoning, PR China), the approval number from the Ethical Committee are [2018]2018-52-2 and [2021]94, and it was performed in compliance with the Declaration of Helsinki Principles. Written informed consent was obtained for all patient samples.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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9. Tables

Due to technical limitations, Table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Expression of SLC25 family in different tumors at mRNA level. A, the expression levels of SLC25 family in 33 cancer types. B, the expression level of SLC25A7 in each cancer.

A

B

Figure 2

Expression of SLC25 family at protein level. A, the expression levels of SLC25 proteins in 10 common cancer types. B, the immunohistochemical map of SLC25A24 protein expression in different cancer tissue.

Figure 3

Expression of SLC25 family in cell lines. A, the expression levels of SLC25 family in each cell line from CCLE. B, the expression level of SLC25A5 gene in different cell lines from CCLE.

Figure 4

Association of SLC25 family with tumor immune cell infiltration. A, the correlation between SLC25 family and tumor-related pathways. Yellow dots represent genes, red dots represent positively correlated pathways and blue dots represent negatively correlated pathways. B, the number of tumor-related pathways in SLC25 family. C, the correlation between SLC25 family genes. D, the maximal correlation

between SLC25s and immune cell infiltration in each cancer tissue. E, the correlation between SLC25 family and immune cell infiltration in all cancer tissue.

Figure 5

Association of SLC25 family expression with prognosis. A, the association between the expression of different genes in SLC25 family and prognosis. B, the Kaplan-Meier survival curves of cervical squamous cell carcinoma and endocervical adenocarcinoma grouped by the overall expression pattern of SLC25A8.

Figure 6

Genetic variation of SLC25 family in pan-cancer. A, the mutation frequency of SLC25s in different cancer tissue. B, the copy number variation of SLC25s in different cancer tissue. C, the mutation status of SLC25s in each cell line.

Figure 8

A, SLC25A4 mRNA expression in STAD, N=23 per group. B, SLC25A23 mRNA expression in COAD, N=30 per group. C, SLC25A7 mRNA expression in STAD, N=56 per group. D, SLC25A7 mRNA expression in COAD, N=13 per group. STAD, stomach adenocarcinoma; COAD, colon adenocarcinoma.

Supplementary Files

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