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学位論文題名	*SLC22A3* that encodes organic cation transporter-3 is associated with prognosis and immunogenicity of human lung squamous cell carcinoma. (有機カチオントランスポーター-3 をコードする *SLC22A3* はヒト肺扁平上皮癌の予後および免疫原性に関連する)
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学位論文内容の要旨

Background:

For decades, lung cancer has consistently ranked the deadliest cancer globally. Lung cancer has two main types: small-cell lung cancer and non-small cell lung cancer (NSCLC). Based on the histological characteristics, NSCLC is further classified as lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LSCC), and others. LSCC accounts for nearly 20% of all lung cancer cases with 5-year relative survival rates of only 24.2% in 2020. In contrast to LUAD, LSCC patients respond poorly to targeted therapy such as therapies for *EGFR*, *KRAS* mutations, and *ALK* rearrangements. Moreover, a large portion of LSCC patients resistant to immune checkpoint therapy. Therefore, a deeper understanding of the molecular abnormalities underlying LSCC is needed to improve the cancer treatment strategies.

Organic cation transporter (OCT)-3 is a bidirectional and polyspecific transporter for cationic molecules including monoamines, metabolites and certain drugs. *SLC22A3*, the gene which encodes OCT-3, has been linked to the prognosis and treatment responsiveness of several types of cancer such as colorectal and cervical cancer. However, its role in LSCC has not been addressed elsewhere. Therefore, our study aimed to elucidate the impact of *SLC22A3* expression on LSCC prognosis and biology.

Methods:

TCGA-LUSC (n = 504) is one of the largest projects in the TCGA program (The Cancer Genome Atlas, National Cancer Institute, USA), exclusively consists of LSCC patients. We retrieved and analyzed gene expression, DNA methylation, and clinicopathological data from TCGA-LUSC. A sample which represents a case in our study is the first sample of the primary tumor. Therefore, normal tissue, later samples of the primary tumor, recurrent or metastatic tumors were excluded. After applying the sample criteria, 501 samples of primary tumors, each of which corresponds to an LSCC case, were included in our analyses.

Using a 5 fragments per kilobase of exon per million mapped fragments (FPKM) cut-off, we

divided LSCC patients into two groups: patients with tumors possessing high and low *SLC22A3* expression (*SLC22A3*-high and *SLC22A3*-low, respectively). Differential methylation position examines the methylation status of CpG sites within the *SLC22A3* gene, including the gene promoter and body.

Prognostic significance was determined through univariate and multivariate Cox analyses for overall survival (OS) and Kaplan-Meier curves for OS and disease-free survival (DFS). We employed the DESeq2 pipeline to explore the differentially expressed genes in the three panels including nCounter Human PanCancer Pathways (CHPP), nCounter Human Immune Profile (CHIP), and nCounter Human PanCancer Progression (CHPPr). Such panels consist of 770, 772, and 770 genes of interest, respectively.

For the pathway analysis, we used gene set variation analysis (GSVA) to investigate the enrichment score (ES) of all the pathways included in The Molecular Signatures Database (MSigDB) Hallmark Gene Set Collection and the cell-type specific signals are markers from the previous study, which includes 24 different cell types.

Validation was carried out in Gene Expression Omnibus (GEO) datasets including GSE74777 (n=107), GSE37745 (n=66), GSE162520 (n=45) and GSE161537 (n=17).

Results: A total of 17.8% LSCCs possess high expression (FPKM ≥ 5) of the *SLC22A3* gene in the TCGA-LUSC cohort. The different expression levels of *SLC22A3* in LSCC were correlated with the methylation status of the *SLC22A3* gene.

Kaplan-Meier OS curves showed that *SLC22A3*-high expression was associated with worse prognoses regardless of chemotherapy and radiation ($p < 0.05$). DFS curves also revealed a substantial difference between *SLC22A3*-high and *SLC22A3*-low individuals, particularly in groups treated solely with radiation ($p < 0.001$). *SLC22A3* expression was a prognostic factor in both univariate (hazard ratio [HR] = 1.82; 95% confidence interval [95%CI] = 1.32 – 2.52; $p < 0.001$) and multivariate (HR = 2.47; 95%CI = 1.65 – 3.71; $p < 0.001$) evaluations. In addition, the 5-year OS rates of patients with *SLC22A3*-low and *SLC22A3*-high LSCC were 51.5% (95% CI: 45.4–58.4%) and 22.9% (95%CI: 13.0–40.6%), respectively. The 5-year DFS rates of *SLC22A3*-low and *SLC22A3*-high LSCC were 52.6% (95%CI = 45.3% - 61.0%) and 35.2% (95%CI = 18.4% - 67.2%), respectively.

t-SNE dimension reduction on CHPP, CHIP, and CHPPr gene matrices suggested possible link between *SLC22A3* expression patterns in LSCC and cancer pathways, cancer-immune interaction, and cancer progression. In CHPP, CHIP, and CHPPr, there were numerous differentially expressed genes between *SLC22A3*-high and *SLC22A3*-low LSCC. Notably, in the *SLC22A3*-high group, many genes encoding immunological checkpoint inhibitory molecules were upregulated, namely: PDCD1, CTLA-4, TIGIT, HAVCR2, and BTLA.

Pathway analysis indicated that *SLC22A3* expression levels were positively correlated with immune-related pathways such as inflammatory response, IL6-JAK-STAT3, complement, allograft rejection, coagulation, IFN-α, and IFN-γ response (adjusted $p < 0.001$) and abundance of infiltrating immune cells in the tumor microenvironment including B-cells, T-cells, and T-cell subpopulations (T helper 1, T gamma delta, CD8+ T, T central memory, T effector memory, and T follicular helper, regulatory T cells), C56-dim natural killer, dendritic cells, macrophages, mast cells, eosinophils, and neutrophils. In addition, *SLC22A3* expression positively correlated with the Hot Oral Tumor (HOT) score, indicating high tumor immunogenicity.

Conclusions: These findings suggest that high expression of *SLC22A3* is associated with poor prognosis and high immunogenicity in LSCC tumors. Understanding the functional implications of *SLC22A3* in LSCC and how it interacts with the immune system may help improve LSCC patient stratification for optimizing immune checkpoint inhibitor therapy treatment, thereby potentially improving outcomes for LSCC patients.

論文審査結果の要旨

1.　学位論文研究テーマの学術的意義

SLC22A3 遺伝子は、オーガニックカチオントランスポーター3 (OCT-3)をコードし、複数のがんの予後と関連していることが報告されているが。肺扁平上皮癌(LSCC)における役割は不明である。LSCC における SLC22A3 遺伝子の関与を明らかにするため、公開データベースであるがんゲノムアトラス-肺扁平上皮癌(TCGA-LUSC)から、遺伝子発現、DNA メチル化、病理学的データを分析した。SLC22A3 の高発現群と低発現群に患者を分け、全生存期間(OS)と無病生存期間(DFS)の予後への影響を検討し、さらに異なるメチル化位置(DMP) 、遺伝子発現の差異、経路分析を行い、複数のデータセットを用いて検証した。SLC22A3 の高発現を示す LSCC 患者は、低発現群に比べて OS と DFS が有意に短かった。また SLC22A3 の発現レベルは、免疫関連経路や腫瘍微小環境における免疫細胞の浸潤と正の相関を認めた。以上のことから、SLC22A3 の高発現は LSCC 腫瘍の予後不良と関連すること、一方で高い免疫原性と関連していることが示唆された。

2.　学位論文及び研究の争点、問題点、疑問点、新しい視点等

本研究は、公開データベースである TCGA のデータを元に、バイオインフォマティクスを用いて肺扁平上皮癌の予後予測因子として、SLC22A3 遺伝子を見いだし、さらに別のコホートを用いて検証まで実施していることは評価できる。またさらに肺扁平上皮癌における SLC22A3 の高発現と炎症反応や免疫関連経路との関連、種々の増殖シグナルとの関連などを、同様にバイオインフォマティクスの手法で明らかにした。一方でこれら一連のデータはすべて in silico に得られたもので記述的な内容に留まっており、実際の臨床検体での OCT3 のタンパク発現は未施行で、他の様々な因子等に対する因果関係に関する in vitro や in vitro での解析による検証はなされておらず、SLC22A3 の肺扁平上皮癌における真の役割に関しては未だ明らかにされていない。本研究では、非常に多くの因子やパスウェイが SLC22A3 の発現に関わる因子として同定されているが、今後は論理的にフォーカスを絞り、肺扁平上皮癌における SLC22A3 遺伝子の役割や機能を明らかにしていくことが望まれる。さらに SLC22A3 の肺扁平上皮癌のバイオマーカーとしての有用性や、機能的解析を踏まえて治療の標的になり得るような研究が推進されることが期待される。

3.　実験及びデータの信頼性

公開されたデータベースを元に解析を実施していること、一般的なバイオインフォマティクスの手法を用いた解析を行っており、複数のコホートを用いて検証を行っていることからデータの信頼性は高いと思われる。

4.　学位論文の改善点、等々

提出された論文の書式、内容、図表、英文は学位論文として十分な水準にある。申請論文は、Translational Lung Cancer Reearch 誌(2023 年、Impact factor ： 4.726)に採択、発行されており、修正、加筆の必要なしと判断した。