# 論　文　内　容　の　要　旨
# Synopsis of Thesis

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| 氏　　名<br>Ｎａｍｅ | 劉　星明 |
| 論文題名<br>Title | Identification of tumor-suppressive miRNAs that target amino acid transporter LAT1 and exhibit anti-proliferative effects on cholangiocarcinoma cells<br>（胆管がん細胞におけるアミノ酸トランスポーターLAT1の発現と細胞増殖を抑制する抗がん作用を持つmiRNAの同定） |

論文内容の要旨

〔目　的(Purpose)〕

L-type amino acid transporter (LAT1, SLC7A5), which preferentially transports large neutral amino acids including most of the essential amino acids, is highly upregulated and associated with poor prognosis in patients with various types of cancer. Due to such pathological significance, LAT1 has been recognized as a novel target molecule in cancer therapy and diagnosis. The first-in-class anticancer drug targeting LAT1, nanvuranlat (developed as JPH203), is currently under clinical evaluation. The first phase II clinical trial of nanvuranlat met the primary endpoint, exhibiting positive clinical efficacy against biliary tract cancer, including cholangiocarcinoma (CHOL). However, while the attempts for the clinical applications of LAT1 inhibitors have been in steady progress, our understanding of the molecular mechanisms responsible for the pathological upregulation of LAT1 in cancer cells is still limited. MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional silencing of specific target genes. Through binding to fully or partially complementary sequences in 3’ UTR of target mRNAs, miRNA induces mRNA degradation or inhibition of translational initiation, thereby playing fundamental roles in various physiological and pathological processes, including tumorigenesis and cancer progression. The present study aims to reveal the novel miRNA-mediated regulatory mechanism for the expression of LAT1 in CHOL cells and explore the potential tumor-suppressive effect of such miRNAs.

〔方法ならびに成績(Methods/Results)〕

The analysis of RNA-seq data from TCGA demonstrated that the level of LAT1 mRNA in CHOL significantly increased comparing with that in normal tissues. By using online target prediction methods, we extracted five candidate miRNAs commonly predicted to regulate the LAT1 expression. Three of them, miR-194-5p, miR-122-5p, and miR-126-3p, were significantly downregulated in CHOL cancer compared to normal tissues. Correlation analysis revealed weak-to-moderate negative correlations between the expression of these miRNAs and LAT1 mRNA in CHOL cancer tissues. We selected miR-194-5p and miR-122-5p for further analyses and confirmed that both miRNAs functionally target 3’UTR of LAT1 mRNA by a luciferase-based reporter assay. Transfection of miR-194-5p and miR-122-5p mimics of significantly suppressed the LAT1 expression at mRNA and protein levels in all the five CHOL cell lines tested. Among them, three cell lines (KKU-055, KKU-100, and KKU-213) were used to further evaluate the effects of these LAT1-targeting miRNAs on cell proliferation and intracellular amino acid levels. Transfection of miR-194-5p and miR-122-5p mimics significantly inhibited the proliferation of the CHOL cell lines in two-dimensional culture and suppressed the growth of KKU-055 cell in spheroid culture. siRNA-mediated knockdown of LAT1 also attenuated the proliferation of CHOL cells, indicating that the anti-proliferative effects of the miRNA mimics, at least partly, can be attributed to their silencing effects on LAT1 expression. As the possible molecular basis underlying the suppression of cell proliferation, HPLC-based quantification of intracellular large neutral amino acids revealed a clear tendency of reduction by the transfection of miR-194-5p and miR-122-5p mimics, with consistent influence on the amino acid-related mTORC1 and GAAC signaling pathways.

〔総　括(Conclusion)〕

We identified two novel LAT1-targeting miRNAs, miR-194-5p and miR-122-5p, and demonstrated their tumor-suppressive function in CHOL cell lines. The pathologically downregulated expression of these miRNAs in tumor tissue may contribute to the overexpression of LAT1, disease progression, and poor clinical outcomes of CHOL patients. It would be interesting to explore the potential of these miRNAs in developing novel therapeutic and diagnostic tools for CHOL, one of the most intractable cancers.

# 論文審査の結果の要旨及び担当者

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## 論文審査の結果の要旨

アミノ酸トランスポーターLAT1は様々な腫瘍組織で高発現しており、がんの治療および診断の新たな標的分子として注目されている。胆管がんを含む幾つかのがん種では、LAT1の高発現が予後不良因子となることも知られている。しかし、LAT1の発現が病態特異的に亢進する分子機構には不明な点が多く残されている。申請者は、低分子非翻訳RNAのマイクロRNA（miRNA）に着目し、複数の標的予測アルゴリズムを併用したLAT1標的miRNAの選定と、The Cancer Genome Atlas（TCGA）データベースに収載のRNA-seqおよびmiRNA-seqデータを用いた発現相関解析を実施した。その結果、胆管がん腫瘍組織で発現が低下し、LAT1の発現と負の相関を示すmiR-194-5pおよびmiR-122-5pを見出した。これらのmiRNAを異所的に胆管がん細胞株に導入したところ、LAT1の発現低下、細胞内遊離アミノ酸量の減少、アミノ酸関連シグナル経路の抑制を引き起こし、細胞増殖が有意に抑制された。以上より、これらのmiRNAの発現低下を機序とするLAT1の発現亢進が、胆管がんの病態進行や予後不良に寄与していることが示唆された。LAT1の発現制御に与る新たな分子機構の解明を通して、がんの病態理解にも貢献するものであり、学位に値すると考える。