論　文　内　容　の　要　旨
Synopsis of Thesis

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論文題名 Title	Upregulation of ATF4 mediates the cellular adaptation to pharmacologic inhibition of amino acid transporter LAT1 in pancreatic ductal adenocarcinoma cells (ATF4の発現上昇は、膵管腺癌細胞におけるアミノ酸トランスポーターLAT1の薬理学的阻害に対する細胞適応を仲介する)

論文内容の要旨

〔目　的(Purpose)〕

The L-type amino acid transporter 1 (LAT1/SLC7A5) is known for its ability to transport a broad spectrum of neutral amino acids, particularly those with large branched or aromatic side chains. Its protein expression exhibits a remarkable specificity for cancer cells, making it an attractive candidate for targeted therapies1. Furthermore, the elevated LAT1 expression correlates with poorer prognoses in various cancer types, including but not limited to pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and biliary tract cancer (BTC). Both pharmacological and genetic inhibition of LAT1 have demonstrated substantial anti-proliferative effects in pre-clinical settings1, underscoring the significant potential of LAT1 as a therapeutic target in cancer treatment. As LAT1 is crucial for supplying cancer cells with numerous essential amino acids, the primary cellular response to LAT1 inhibition is mediated through pathways responding to amino acid deprivation. However, the potential cellular adaptation to LAT1 inhibition remains poorly understand. In this present study, we explored the cellular adaptation to LAT1 inhibition by using nanvuranlat (a highly selective inhibitor of LAT1) in cell lines from pancreatic ductal adenocarcinoma (PDAC).

〔方法ならびに成績(Methods/Results)〕

To elucidate potential factors involved in the adaptive response to nanvuranlat, we performed a proteomic analysis on MIA PaCa-2 cells (a human PDAC cell line) treated with or without nanvuranlat. Using ingenuity pathway analysis, we predicted the upstream regulators that might be activated or in activated in nanvuranlat-treated cells. Among those regulators, we selected activating transcription factor 4 (ATF4) for further investigation, given its noted role in reducing the sensitivity of cancer cells to amino acid starvation.

We confirmed the upregulation of ATF4 following nanvuranlat treatment in MIA PaCa-2 and HPAC cells (another human PDAC cell line). Then, we confirmed the activation of general control nonderepressible 2 (GCN2) and eukaryotic initiation factor 2α (eIF2α). The GCN2-eIF2α pathway is well-recognized for upregulating ATF4 protein expression in response to amino acid starvation. We found that siGCN2 and ISRIB (an inhibitor for eIF2α) suppressed nanvuranlat-induced upregulation of ATF4, suggesting the involvement of the GCN2-eIF2α pathway in the upregulation of ATF4 following nanvuranlat treatment. Then, we showed that the combination of ATF4-knockdown and nanvuranlat significantly suppressed cell proliferation *in vitro* compared to single-agent. Furthermore, we found that ATF4-knockdown increased the sensitivity of cancer cells to nanvuranlat.

〔総　括(Conclusion)〕

Our findings indicate that ATF4 upregulation occurs upon LAT1 inhibition. We confirm the activation of GNC2-eIF2α pathway as a mechanism to induce ATF4 upregulation in nanvuranlat-treated cells. Importantly, our data demonstrate that depleting ATF4 sensitizes these cancer cells to LAT1 inhibition. Thus, our results suggest that inactivating ATF4 could be a promising strategy to counteract the compensatory mechanisms induced by LAT1 inhibition and to amplify the therapeutic impact of LAT1 inhibitors.

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

L型アミノ酸トランスポーター1（LAT1；SLC7A5）は、がん治療の標的として種々の研究がなされているが、その薬理学的阻害に対する細胞の適応応答については、詳細な検討はされていない。本研究は、高親和性LAT1阻害薬であるnanvuranlatを使用し、LAT1阻害に対する適応応答を解析したものである。LAT1阻害により、アミノ酸欠乏に対する応答に関わるストレス誘発型転写因子ATF4の活性化が生じることをプロテオーム解析により明らかにしている。さらに、この活性化は翻訳開始を調節するGCN2-eIF2α経路を介するものであることを明らかにし、LAT1阻害への細胞の適応応答においてATF4が果たす役割を示した。加えて、LAT1阻害とATF4ノックダウンを併用することで、それぞれの単独よりも細胞増殖抑制効果が増強されることを明らかにし、ATF4の上方制御がnanvuranlatの細胞増殖抑制効果を低減する可能性を示唆している。本研究は、LAT1阻害による腫瘍細胞の適応応答の一端を明らかにするとともに、その解除法を提示しており、学位に値すると考える。