論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	周　新宇
論文題名 Title	Inhibition of amino acid transporter LAT1 in cancer cells suppresses G0/G1-S transition by downregulating cyclin D1 via p38 MAPK activation （がん細胞におけるアミノ酸輸送体LAT1の阻害はp38 MAPKの活性化を介したサイクリンD1のダウンレギュレーションによりG0/G1期からS期への移行を抑制する）

論文内容の要旨

〔目　的(Purpose)〕

L-type amino acid transporter 1 (LAT1, SLC7A5) mediates the transport of large neutral amino acids across the plasma membrane. Amino acids are utilized in cells for various purposes, including biosynthetic reactions such as protein synthesis, bioenergetic reactions such as ATP synthesis, and also as signaling molecules that activate nutrient sensing systems to control the cell metabolism. LAT1 expression is limited in normal tissues, whereas remarkably upregulated in various types of cancers. Furthermore, in such cancers, the higher LAT1 expression levels are often associated with aggressive disease phenotypes and poor clinical outcomes. Due to its pathological upregulation and contribution to the disease progression in various cancer types, LAT1 has been gaining increasing attention as a novel target for cancer therapy. Nanvuranlat (Nanv, JPH203) is a selective LAT1 inhibitor that has been developed as a novel class of anti-cancer agent and currently under clinical evaluation. Previous studies have demonstrated that Nanv drastically inhibits the uptake of large neutral amino acids and suppresses the growth of cancer cells of various tissue/organ origins both *in vitro* and *in vivo*. It has also been shown that the pharmacological inhibition of LAT1, as well as the suppression of LAT1 gene expression, increases cells at G0/G1 phase, suggesting the cell cycle arresting effects of Nanv. However, at which step and via which mechanisms Nanv perturbates the cell cycle progression of cancer cells is still unclear. To improve our understanding of the pharmacological activity of LAT1 inhibitors as anti-cancer drugs, we investigated the molecular mechanisms underlying the suppression of cancer cell proliferation by LAT1 inhibition, specifically focusing on the cell cycle.

〔方法ならびに成績(Methods/Results)〕

We selected pancreatic cancer cell lines (MIA PaCa-2, AsPC-1, and PANC-1) to investigate the effects LAT1 inhibition on the cell cycle because the anti-proliferative effects of Nanv have been most extensively studied. Cells were treated with a serum-reduced medium containing 1% FCS for 24h to be synchronized at the growth factor-dependent restriction point (R), the primary cell cycle checkpoint in G0/G1 phase. Then, the cells were released in a culture medium containing 10% FCS in the presence or absence of Nanv. A time course analysis of cell cycle by flow cytometry revealed that Nanv potently blocks the G0/G1-S transition of cancer cells after the release. At the molecular level, cyclin D1, a cell cycle regulator in the G0/G1 phase, was transiently increased in the absence of Nanv, which precedes the increase of cells in S phase. In contrast, Nanv drastically suppressed the increase of cyclin D1 in all the three cell lines tested. Using MIA PaCa-2 cells, we found that the decrease in the total amount of cyclin D1 induced by Nanv is accompanied by an increased phosphorylation level of cyclin D1, suggesting the enhanced phosphorylation-dependent proteasomal degradation. Consistently, co-treatment of proteasome inhibitors with Nanv restored the expression of cyclin D1 and reverted the cell cycling arresting effect of Nanv. As the mechanism responsible for the increased phosphorylation of cyclin D1, we found a sustained phosphorylation of MAP kinase p38 in the presence of Nanv. Further analyses through the siRNA mediated gene knockdown of four p38 isoforms demonstrated that p38α is mainly expressed and phosphorylated by Nanv in the pancreatic cancer cell lines tested, thereby predominantly contributing to the cyclin D1 dysregulation. In Nanv-treated MIA PaCa-2 cells, an activation of canonical p38 upstream kinases, MKK3 and MKK6, rather than the decreased activities of p38 phosphatases was suggested to be involved in the activation of p38α. Finally, Nanv significantly suppressed the growth of MIA PaCA-2 xenograft tumor model *in vivo*, with a concomitant activation of MKK3/6, p38, and a decline of cyclin D1 amount.

〔総　括(Conclusion)〕

The inhibition of LAT1 with Nanv arrested the cell cycle at G0/G1-S transition in pancreatic cancer cell lines. This effect was mediated by the activation of p38α and phosphorylation-dependent proteasomal degradation of cyclin D1. These findings contribute to delineating the pharmacological activities of LAT1 inhibitors as anti-cancer agents, providing valuable implications for their clinical development. Verifying the findings of this study in other types of cancer cells in future studies will establish the blockade of G0/G1-S transition as a fundamental pharmacological effect of LAT1 inhibitors. This study also provides significant insights into the molecular basis of previously proposed, yet uncharacterized, EAAs-mediated checkpoint in G0/G1 phase.

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

大型中性アミノ酸の選択的輸送を担うアミノ酸トランスポーターLAT1は、様々な腫瘍組織において発現が亢進しており、その高発現は予後不良因子となることが知られている。LAT1阻害薬nanvuranlat（Nanv, JPH203）は、腫瘍細胞へのアミノ酸取込みの遮断を機序とした、新たな抗悪性腫瘍薬として開発が進められてきた。先行研究により、Nanvが細胞周期停止作用を有することが示唆されてきたが、その詳細は不明であった。申請者は、膵臓がん細胞株を用いて、NanvがG0/G1期からS期への移行を強力に阻害することを示した。その分子機構として、p38α MAPキナーゼの持続的活性化と、それに伴う細胞周期制御因子cyclin D1のリン酸化依存的なプロテアソームにおける分解の促進を見出した。また、担がんマウスモデルにおいても同様の機序の存在が示唆された。抗悪性腫瘍薬として開発が進むNanvの薬理作用の理解に貢献すると同時に、アミノ酸依存的な新たな細胞周期制御の分子機構についても重要な知見を与える成果であり、学位に値すると考える。