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論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	WONGTHAI PRINTIP
論文題名 Title	Boronophenylalanine, a boron delivery agent for boron neutron capture therapy, is transported by $ATB^{0,+}$, LAT1 and LAT2 （ホウ素中性子捕捉療法に用いるホウ素化合物*p*-ボロノフェニルアラニンの細胞への取り込みはアミノ酸トランスポーター$ATB^{0,+}$、LAT1および LAT2 が担う）

論文内容の要旨

〔目 的(Purpose)〕

The efficacy of boron neutron capture therapy relies on the selective delivery of boron-carriers to malignant cells. *p*-Boronophenylalanine (BPA), a boron delivery agent, has been proposed to be localized to the cells via transporter-mediated mechanisms. Alterations of transporter expression in tumor cells, an adaptation to alter tumor metabolism, offer opportunities for selective drug delivery. In this context, aromatic amino acid transporters up-regulated in tumor cells are expected to play an important role in the delivery of BPA, a phenylalanine analogue. To reveal the mechanisms of BPA uptake by tumor cells, we screened aromatic amino acid transporters to search for BPA transporters.

〔方法ならびに成績(Methods/Results)〕

Methods:

The human cRNA of aromatic amino acid transporters (LAT1, LAT2, LAT3, LAT4, TAT1, $ATB^{0,+}$ and B^0AT1) were synthesized *in vitro* and functionally expressed in *Xenopus* oocytes. The oocytes expressing aromatic amino acid transporters were screened for BPA uptake and kinetic parameters for BPA were determined. The roles of the BPA transporters were investigated in cancer cell lines in the presence and absence of sodium. The membrane fraction of cancer cell lines was used to determine the expression of transporters by western blot analysis. siRNA of LAT1 was used to clarify the contribution of LAT1 to BPA uptake in a breast cancer cell line MCF-7. For quantitative assessment of BPA uptake, a pre-column derivatization-HPLC was used throughout this study.

Results:

Among aromatic amino acid transporters examined, $ATB^{0,+}$, LAT1 and LAT2 were found to transport BPA with K_m values of 137.4 ± 11.7, 20.3 ± 0.8, and 88.3 ± 5.6 μM, respectively. LAT1 and $ATB^{0,+}$ are Na^+-independent and Na^+-dependent transporters, respectively, and known for their relevance to malignant tumors. By mean of western blot analysis on tumor cell lines, the expression of LAT1 was none in FLC-4, medium in MCF-7 and MIA Paca-2 and high in HeLa S3 and T3M4 cells. The expression of $ATB^{0,+}$ was detected in MCF-7 and T3M4 cells. Uptake experiment in cancer cell lines revealed that the LAT1 protein amount was the major determinant of BPA uptake at 100 μM, whereas the contribution of $ATB^{0,+}$ became significant at 1000 μM, accounting for 20–25 % of the total BPA uptake in MCF-7 breast cancer cells.

〔総 括(Conclusion)〕

LAT1, LAT2 and $ATB^{0,+}$ transport BPA at affinities comparable with their endogenous substrates, suggesting that they could mediate effective BPA uptake *in vivo*. The high and low affinities of LAT1 and $ATB^{0,+}$, respectively, differentiate their roles in BPA uptake. $ATB^{0,+}$, as well as LAT1, could contribute significantly to the tumor accumulation of BPA at clinical BPA dosage.

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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

ホウ素中性子捕捉療法(BNCT)は、がん細胞に取り込ませたホウ素（^{10}B）と中性子線との核反応で発生する粒子線によりがん細胞を障害するものであり、がん細胞特異的に効率良く^{10}Bを取り込ませることが、治療の有効性の重要な要因となる。本研究は、BNCTに使用されるホウ素化合物p-ボロノフェニルアラニン（BPA）のがん細胞への取り込み機構を明らかにするため、芳香族アミノ酸を輸送するトランスポーターを検討し、$ATB^{0,+}$、LAT1、LAT2がBPAを輸送することを明らかにした。このうち$ATB^{0,+}$とLAT1ががん細胞へのBPA送達を担い、LAT1が主要な取り込み経路であるが、高濃度域では$ATB^{0,+}$の寄与も有意となることが示された。この結果は、臨床用量では、がん細胞のBPAの取り込みは$ATB^{0,+}$とLAT1の両者を介していることを示唆している。本研究は、がん細胞へのBPA取り込み機序を明らかにし、より有効性の高い化合物創製への基盤となるものであり、学位の授与に値すると考えられる。