L-Type Amino Acid Transporters As Targets For Cancer Diagnosis And Therapeutics

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Molecular biology has made it possible to identify membrane transporter molecules that transport hydrophilic endogenous and exogenous compounds across cell membranes. In oncology, there are several possibilities on transporters relevant to new diagnostic methods and therapeutics such as low molecular anti-cancer agents, monoclonal antibodies and gene therapy.

L-type amino acid transporters (LATs) and their functions

In general, cell growth in cancers is more rapid than that in normal cells. For their rapid growth, essential amino acids are inevitable. We identified oncofetal 12-transmembrane L-type amino acid transporter named LAT1 (1) and normal cell type LAT2 (2). LAT1 transports amino acids like leucine, phenylalanine, tyrosine, isoleucine, tryptophane and valine in a higher affinity manner than LAT2. LAT1 and LAT2 are corresponding to classical NEM (*N*-methylmaleimide)-insensitive type of system L (system L1) and belong to SLC7 gene family. In addition, we have recently identified a new family of system L transporters LAT3 and LAT4 corresponding to NEM-sensitive type of system L (system L2) in SLC43 family (3). It has turned out that LAT3 is a cancer type whereas LAT4 is a normal cell type.

L-type amino acid transporters (LATs) as molecular targets for cancer diagnosis

LAT1 was highly expressed in several types of human malignant tumors including colorectal carcinomas, gastric cancers, pancreatic cancers, mammary gland carcinomas, renal carcinomas, esophageal carcinomas, lung cancers and brain tumors. On the other hand, LAT3 was expressed differently from LAT1 in other types of cancers like hepatoma and prostate cancer. Thus, to increase the efficacy of chemotherapeutics, types of LAT expression should be identified immunohistochemically prior to their inhibitor application to respective patients.

L-type amino acid transporters (LATs) as molecular targets for novel anticancer agents

We synthesized and screened new compounds, KYT0193, KYT0206 and KYT0213 using stable cell lines of human LAT1 and LAT2. As new inhibitors, we propose suitable drug candidates. By *in vitro* experiments, concentration-dependent inhibition of leucine uptake by amino acid related compounds are obtained using LAT1 and LAT2 cell lines. KYT0193 was most potent to inhibit [14C]-L-leucine uptake, whereas KYT0206 was relatively LAT1-specific, and KYT 0213 was LAT2-specific inhibitor. We tested *in vivo* effects of KYT0193 given subcutaneously using nude mice inoculated by T24 cells originated from human urinary bladder cancer. KYT0193 significantly suppressed the tumor growth. We also determined survival rate of ICR mice injected S180 mouse sarcoma cells intraperitoneally. KYT0193 as well as LAT1 antisense oligo elongated the rate of survivals significantly. From these results, LAT1 would be a new target molecule for creating novel anticancer agents.

Monoclonal antibodies against L-type amino acid transporters (LATs) as new immuno-therapeutics

The strategy of Herceptin whose molecular target is epidermal growth factor receptor protein can be applied to LAT1 and LAT3. We are trying to produce monoclonal antibodies against extracellular epitopes of these LATs using "Xenomouse".

Gene therapy: knock-down of cancer-specific L-type amino acid transporters (LATs)

We have already identified positive effects of antisense for LAT1 on inhibition of cancer cell growth. Therefore, RNAi can be utilized similarly to antisense as gene therapeutics.

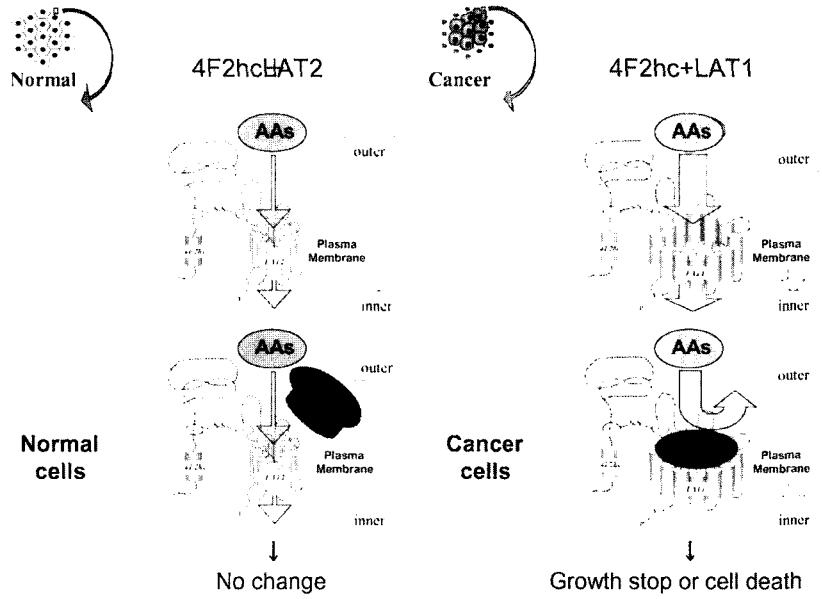
Future perspective

Rapid progress on transporter research includes new transporters in intracellular organelles and new binding proteins to transporter molecules which regulate functions of transporter catalytic units. These new molecules may clarify disease mechanisms and become reasonable molecular targets for new drugs. Thus, precise intracellular mechanisms of amino acid starvation could be clarified. The combination of diagnosis with therapeutics enables not only to increase the efficacy but also to decrease their side effects. In addition, single nucleotide polymorphism (SNP) is an important issue to be further investigated in transporter research, since racial and regional individual variations in drug action exist. This kind of SNP analysis may clarify mechanisms of different drug actions in individuals and increase their quality of life.

References

(1) *J. Biol. Chem.* 273: 23629-23632, 1998. (2) *J. Biol. Chem.* 274(28): 19745-19751, 1999. (3) *J. Biol. Chem.* 278 (39): 43838-43845 2003.

Starve-out Strategy of Cancer cells



Strategy of Anti-Cancer Therapeutics

Malignant Transformation

Activation of Oncogene

Inactivation of Tumor suppressor gene

Growth acceleration
Metastasis

Essential amino acids

Increase of LAT1/3 expression and function
***Final Common Path* of the cancer>**

Anti-LAT1/3 therapy
Low MW antisense/RNAi monoclonal Ab)