Up-regulation of LAT1 during antiandrogen therapy contributes to progression in prostate cancer cells

（抗アンドロゲン療法に伴う LAT1 の発現増加は、前立腺癌細胞の増殖に寄与する）

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Abstract

Purpose

Cancer cells require massive amounts of amino acids for survival. L-type amino acid transporter 1 (LAT1) transports essential amino acids, including leucine, which trigger the downstream m-TOR pathway. The association between androgen receptor (AR) and LAT1 and the association between LAT1 expression and acquisition of castration resistance were examined.

Materials and Methods

Western blotting and real-time polymerase chain reaction were used to study protein and mRNA expressions. SiRNA was used to knockdown target genes. 92 prostate biopsies specimen of patients who underwent androgen deprivation therapy (ADT) were used for immunohistochemical analyses. Cox hazard proportional models and the Kaplan-Meier method were used for statistical analyses.
Results

LAT1 was highly expressed in hormone-resistant prostate cancer cell lines. Knockdown of LAT1 in LNCaP and C4-2 cells significantly suppressed cell proliferation, migration, and invasion. SiRNA of AR or blocking of AR through bicalutamide (10µM) or MDV3100 (10µM) significantly increased LAT1 expression (P<0.01). Treatment with DHT (0.1-10 nM) reduced LAT1 expression in a dose-dependent manner (P<0.01). Bicalutamide/MDV3100 plus SiLAT1 synergistically suppressed prostate cancer cell proliferation compared to single inhibition of AR or LAT1 (P<0.01). The high LAT1 expression correlated with significantly shorter PSA recurrence free survival in patients receiving ADT (P<0.0001). LAT1 expression (HR 3.56, P = 0.0133) was an independent predictor of castration resistance on multivariate analysis.

Conclusions

The current data may provide a novel mechanism to acquire castration resistance through activation of amino acid transporter LAT1.

Introduction

Prostate cancer (PC) is one of the most commonly diagnosed cancers in men.(1, 2)
Since the historical discovery of Dr. Huggins, androgen deprivation therapy (ADT) has been the mainstay of therapy for locally advanced or metastatic PC.(3) Despite the early response to ADT, the majority of patients with advanced disease progress and become refractory to ADT due to emergence of androgen-independent PC cells.(2, 4-6) Although several mechanisms have been proposed, the androgen receptor (AR) plays a central role in the development of CRPC through genomic amplification, ligand independent activation, ETS Related Gene (ERG) fusion, and splicing variants.(7-10)

Cancer cells take up massive amounts of amino acids for survival. L-type amino acid transporter 1 (LAT1) is a member of the solute carrier (SLC) family and transports neutral amino acids, including essential amino acids, into cells in a Na⁺-independent manner.(11) A high level of LAT1 expression has been observed in a wide variety of malignant cells, while normal cells showed limited expression of LAT1, with the only expression reported in the vascular endothelial cells of the blood-brain barrier and syncytiotrophoblastic cells of the placenta.(11) Therefore, it has been suggested that LAT1 plays an essential role in malignant tumor growth by promoting uptake of essential amino acids. Recent evidence has also suggested that LAT1 regulates mammalian target of rapamycin complex 1 (mTORC1), which requires intracellular amino acids such as leucine for its activity.(12) However, the role of LAT1 in the
androgen receptor-mediated pathway remains to be investigated.

In this study, we investigated the regulation of LAT1 by androgen receptor and studied functional contribution of LAT1 in castration resistant prostate cancer.

Materials and Methods

Reagents and antibodies

Lipofectamine RNAiMax reagent, siLAT1 (Stealth siRNAs HSS112004, HSS112005, HSS188571), siAR (Stealth siRNAs, HSS100619, HSS179972, HSS179973), and Stealth RNAi siRNA Negative Control Med GC Duplex #3 were purchased from Invitrogen (Burlington, ON, Canada). Anti-LAT1 (Trans Genic Inc.; KE023), and anti-AR (AR441, ab9474) were obtained from Abcam (Cambridge, UK).

Anti-phosphorylated AKT (Ser473) (587F11), anti-Phospho-4E-BP1 (Thr37/46) (236B4), and anti-Phospho-p70 S6 Kinase (Thr389) (108D2) were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-GAPDH was purchased from Applied Biosystems (Foster, CA, USA).

PC tissue specimens

Total of 92 PC tissue specimens (92 biopsy specimens subsequently treated with anti-androgen therapy) obtained at Chiba University hospital were used for the analysis.
Histopathologic analysis of the tissues was performed according to the World Health Organization criteria by the Department of Pathology, Chiba University Hospital. All patients had PC that was histologically confirmed, and tumor samples were checked to ensure that tumor tissue was present in the specimen.

Cell culture and transfection

LNCaP, C4-2, PC3, and DU145 cell lines, derived from human PCs, were obtained from the RIKEN BRC CELL BANK (Tsukuba, Ibaragi, Japan). The PC cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and maintained in an incubator with a humidified atmosphere of 95% air and 5% CO2 at 37 °C. For steroid-free conditions, phenol red-free RPMI-1640 medium was used with charcoal-stripped FBS (CS-FBS). PC cells were transfected with DNA and/or siRNA using Lipofectamine 2000 and/or Lipofectamine RNAiMax reagent (Invitrogen), according to the manufacturer’s instructions.

Evaluation of mRNA expression

Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer’s instructions. First-strand complementary DNA (cDNA)
was synthesized using the ImProm-II™ Reverse Transcription System with random primers (Promega, Tokyo, Japan). Real-time RT-PCR was performed using an ABI™ 7300 Real-Time PCR System. PCR reactions were performed in a final volume of 25 μl of a reaction mixture of SYBR® Green PCR Master Mix. Primer sequences used for PCR amplification were: LAT-1 Forward: AGGAGCCTTCCTTTCTCCTG, LAT-1 Reverse: CTGCAAACCCTAAGGCAGAG, AR Forward: CCTGGCTTCCGCAACTTACAC, AR Reverse: GGACTTGTGCATGCGGTACTCA. Relative mRNA expression levels were determined by comparison to the GAPDH internal control and plotted as a ratio to GAPDH expression values.

Western blot analysis

Protein expression was determined by immunoblotting using the specific antibodies mentioned above. Protein levels were normalized to GAPDH expression. The experiments were performed according to the protocol described previously.(13) Signal detection was achieved with the ECL Prime Western Blotting Detection Reagent (GE Healthcare Bio-Sciences, Princeton, NJ) and visualized using LAS-4000 mini (FUJIFILM, Tokyo, Japan).
Immunohistochemistry (IHC) was performed on 4-μm-thick sections of paraffin-embedded specimens using goat anti-LAT1 polyclonal antibody according to the protocol described previously. (13) To quantify the state of LAT1 protein expression in these components, each mean percentage of LAT1-positive tumor cells was determined in at least five random fields at ×400 magnification in each section. The intensity of the LAT1 immunoreaction was scored as follows: 0, no staining; 1+, very weak; 2+, moderate; 3+, strong; and 4+, very strong. The staining intensity was scored to produce each LAT1 IHC score. Pathological evaluations were performed under consultation of pathologist Jun Matsushima. These judgments were made by two independent investigators (S.S. and X. M.) both of whom were blinded to the patients’ clinical status.

Growth assay

Cells were trypsinized, and equal numbers (4×10^3 cells) were seeded in a 96-well culture plate for all transfection conditions. After 24 h, the media were changed for fresh complete media (10% FBS), and all sets of cells were transfected with siRNA using Lipofectamine RNAiMax reagent (Invitrogen), according to the manufacturer’s instructions, and left for 4 days. At each indicated time point, cells were trypsinized and
Patient selection and clinical variables

92 biopsy specimens from patients who underwent CAB (combined androgen blockade) as first-line therapy for PC at Chiba University Hospital were retrospectively analyzed. The prognostic values of LAT1 expression and other clinical factors were evaluated in association with prostate specific antigen (PSA) progression-free survival. PSA failure was defined according to the definition of The Prostate Cancer Clinical Trials Working Group 2 (PCWG2): a rising PSA greater than 2 ng/mL higher than the nadir; the rise has to be at least 25% over the nadir; and the rise has to be confirmed by a second PSA at least three weeks later.

Ethical approval

Study was approved by institutional review board (IRB) # 408 and written informed consents were obtained from the patients.

Statistical analysis

Univariate and multivariate Cox proportional models and the Kaplan-Meier method
were used for statistical analyses. Student’s t-test, the chi-square test, and Wilcoxon’s signed rank test were used to assess the associations of LAT1 expression and other clinical variables. Statistical computations were carried out using JMP 11.0.0 (SAS Institute, Cary, NC, USA). Significance was set at P< 0.05.

Results

The expression of LAT1 in PC tissues and the correlation between LAT1 and clinical variables

Protein expression of LAT1 was examined in PC patients by immunohistochemistry (IHC) of prostate specimens. Representative IHC results of biopsy specimen for LAT1 protein in normal prostate tissue and PC tissue are shown in Figure 1. A positive immunoreaction for LAT1 was detected in the membrane, together with the cytoplasm. Strong LAT1 immunoreactions were detected in cancer lesions (black arrow head), whereas noncancerous lesions showed negative or weak immunostaining (white arrow head) (Figure 1A, B). LAT1 expression tends to increase from gleason score (GS) 3 to 5 (Figure 1C, D). Although negative correlation of AR and LAT1 were observed in some lesion, no clear trends between LAT1 and AR (nor PSA) were observed overall (Supplementary figure 1-3).
Next, protein expression levels of LAT1 in biopsy specimen of patients who underwent ADT were studied. Specimens were divided into two groups based on LAT1 and nucleus AR staining (median intensity score (IS) of 3). In biopsy specimens of ADT patients, high LAT1 expression showed significantly shorter time to CRPC than those with low LAT1 expression ($P<0.0001$) (Figure 2A). Although high AR expression group tend to show short time to CRPC, AR expression did not correlated with PSA recurrence free survival ($P=0.0591$) (Figure 2B). When combined LAT1 and AR expression, high AR/ high LAT1 group showed the worst PSA recurrence free survival, followed by high LAT1/ low AR group. High AR/ Low LAT1 and Low AR/ Low LAT1 groups showed similar favorable prognosis (Figure 2C).

Evaluation of LAT1 protein expression in PC-derived cell lines

To investigate basal protein expression of LAT1, Western blot analysis was performed using three PC-derived cell lines (LNCaP, C4-2, PC-3,DU145) (Figure 3A). A significant increase in LAT1 protein expression was observed in androgen-independent C4-2 cells compared to androgen-sensitive LNCaP cells. LAT1 was highly expressed in other androgen-independent AR-negative PC-3 and DU145 cells. Expression analysis indicated that both transcription and translation products of this molecule were highly
expressed in androgen-independent cell lines (Figure 3B).

Establishment of LAT1 knockdown cells

To obtain transient LAT1 knockdown transfectants, the LAT1 siRNA (siLAT1#1-2) plasmid and the negative control siRNA (Nega) plasmid were used. LAT1 mRNA and protein expression was significantly lower (<80%) in siLAT1-transfected cells than in Negative-transfected cells in C4-2 cells and LNCaP Cells (Figure 3C, D, E and F).

LAT1 knockdown inhibited cell growth, migration, and invasion

To investigate the anti-proliferative effects in siLAT1-transfected cells, cellular growth was monitored for 4 days. The siLAT1-transfected cells showed a significant decrease in cellular growth compared with Nega-transfected cells in LNCaP cells and C4-2 cells (Figure 4A and B). SiLAT1-transfected cells also showed significant decreases in cellular migration and invasion compared with Nega-transfected cells in LNCaP and C4-2 cells (Figure 4C, D, E and F).

LAT1 knockdown inhibited m-TOR pathway

In order to investigate the effect of LAT1 knockdown on the m-TOR pathway, siLAT1 were transfected in C4-2 cells, and the phosphorylation status of downstream
m-TOR-related proteins was studied. SiLAT1 inhibited phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and Eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) (lane 72h), while phosphorylation of AKT was not affected by siLAT1 (Figure 5A).

AR knockdown increased LAT1 expression

In order to study the association of AR and LAT1, siAR were transfected in LNCaP and C4-2 cells. Knockdown of AR significantly increased protein and m-RNA levels of LAT1 in LNCaP and C4-2 cells (Figure 5B and C).

Bicalutamide and MDV3100 increased LAT1 expression in the presence of dihydrotestosterone (DHT)

Next, the effect of DHT on LAT1 expression was examined. DHT decreased expression of LAT1 in a dose-dependent manner (DHT 0 to 10nM), while bicalutamide increased LAT1 expression (protein and mRNA levels), even in the presence of DHT (Figure 5D). The effects of anti-androgens to increase LAT1 expression were similar for bicalutamide and MDV3100 (Figure 5E) compared to DHT-only treated cells. LAT1 expression was also assessed in the serum-free condition (5% charcoal filtered FBS),
resembling the castrated condition, and LAT1 expression increased in a time-dependent manner (24 hours to 7 days), along with time-dependent decreased expression of AR (Figure 5F).

Knockdown of AR and LAT1 significantly inhibited cellular growth in LNCaP and C4-2 cells

Since blocking of AR increased LAT1 expression, the effect of dual inhibition of AR and LAT1 using bicalutamide and MDV3100 in combination with siLAT1 was studied in LNCaP and C4-2 cells (Figure 6). The inhibitory effect of cellular growth mediated by bicalutamide or MDV3100 was similar to that of LAT1 knockdown in both LNCaP and C4-2 cells. Dual inhibition of AR (through bicalutamide or MDV3100) and LAT1 significantly inhibited cellular growth compared to single inhibition by AR or LAT1. Interestingly enough, cells treated with anti-androgen and siLAT1 almost completely lost their ability to promote cellular growth (Figure 6A-D) in both LNCaP and C4-2 cells.

In order to study the clinical factors associated with LAT1 expression, high and low LAT1 groups were compared (Table 1). High LAT1 correlated with higher ALP
(P=0.023), PSAD (P=0.03), PSA nadir (P=0.048), cT stage (P=0.0001), and GS (P<0.0001). High LAT1 also correlated with lower age (P=0.001), shorter PSA and TST time to nadir (P=0.003 and P=0.001, respectively) (Table 1).

Furthermore, when assessing the risk of PSA recurrence, on multivariate analysis, LAT1 expression remained as a significant prognostic factor for PSA recurrence under ADT (HR 3.56; P = 0.013), together with GS (HR 1.98; P=0.042) (Table 2).

Discussion

The current data indicated upregulation of LAT1 under castrated condition in PC cells. Knock down of AR or treatment with anti-androgens, such as bicalutamide or MDV3100, showed increased LAT1 expression, while treatment with DHT reduced LAT1 expression. Overexpression of LAT1 in patients with ADT was associated with significantly shorter time to CRPC. These data indicate the contribution of the amino acid transporter LAT1 in the adaptation under castrated condition in PC cells. Since blocking AR increased LAT1 expression, dual inhibition of LAT1 and AR may have therapeutic significance in CRPC patients.

At this stage, the mechanism for the negative regulation of LAT1 by androgen receptor signals is not still clear, whether it is a direct interaction of the androgen receptor with LAT1 or indirect regulation through bypassing signals. Wang et al.
reported that LAT1 is regulated by the amino acid stress pathway and activating transcription factor4 (ATF4) mediated transcription.\(^{(14)}\). They demonstrated upregulation of ATF4 under the leucine-free condition, which stimulated transcription of LAT1 in AR-positive LNCaP cells and AR-negative PC-3 cells. In the present analysis, blocking AR significantly increased LAT1 expression with leucine-containing normal medium, in AR specific manner, indicating current mechanism is distinct from the ATF4-mediated pathway. Classically, direct interaction of AR is known in PTEN and Fork-head box protein A1 (FOXA1). \(^{(13, 15, 16)}\) FOXA1 supports AR function by opening up chromatin, and thus SiRNA of FOXA1 caused a significant reduction of AR activation.\(^{(13)}\) In the case of LAT1, knockdown of LAT1 did not have any effect on AR expression or activation. The only expression change in LAT1 occurred when AR was knocked down or blocked by anti-androgen. Thus, LAT1 seems to be a downstream signal of AR, but direct interaction or mutual regulations are to be determined.

In the current study, high LAT1 expression was an independent risk factor for PSA recurrence during ADT. High LAT1 expression was related to high GS, cT stage, PSAD, PSA nadir and short time to nadir for PSA/Testosterone (TST). The correlation between LAT1 and GS was in accordance with the previous report by Segawa et al.\(^{(17)}\) Regarding time to PSA or TST nadir, our group reported that patients with high nadir
TST or PSA require a shorter time to reach nadir than low nadir patients, which correlated with poor prognosis (18). In the current results as well, high LAT1 patients showed higher nadir PSA (P=0.048) and relatively higher nadir TST (P=0.088), that may affect the prognosis of the ADT patients.

In relation with AR and amino acid transporter, Wang et al., reported positive regulation of LAT3 by AR in prostate cancer cells. They indicated neoadjuvant hormonal therapy significantly decreased LAT3 expression. In combination with our data, one of the possible mechanisms may be that hormonal therapy decrease LAT3, while increase LAT1 during adaptation in castrated condition to acquire essential amino acids into PC cells (19).

Another reason for early recurrence in high LAT1 patients may be the contribution of AR-independent survival signals including the m-TOR pathway. In the present data, in accordance with previous reports (14, 20, 21), knockdown of LAT1 inhibited downregulation of the mTORC1 pathway through inhibition of phosphorylation of S6K1 and 4EBP1.

These mechanisms may contribute not only to hormone naïve prostate cancer treated with first-line ADT, but also for CRPC patients receiving MDV 3100 or abiraterone, which significantly block the AR/TST-mediated pathway. The presence of association
between LAT1 expression and resistance to novel AR targeted drugs will be analyzed further in the future.

As limitations, overexpression of LAT1 was first tried in order to study the functional significance of LAT1. However, no remarkable functional difference was seen (Supplementary figure 4A), which was in accordance with a previous report that only used SiRNA, ShRNA, or LAT1 inhibitor BCH, but not an overexpression system.(14, 22, 23) Second, the precise mechanism of how AR negatively regulates LAT1 is not still clear. Effect of LAT1 knock down seems to be more prominent in AR positive cells compare to AR negative cells (Supplementary figure 4B, C). We are currently pursuing co-immunoprecipitation and a chip assay to determine the possibility of direct interaction between LAT1 and AR. It is not still clear whether AR directly regulate LAT1 expression or as a result of adaption of PC cells under blockade of AR mediated pathway. Third, no significant negative correlation between LAT1 and AR were observed in clinical specimens. This may represent presence of multiple regulatory pathways of LAT1 in microenvironment including ATF4 and c-MYC (14, 24). Fourth, the study cohort used for determination of LAT1 and clinical factors was small, and the study was retrospective. A large, prospective study will clarify the precise contribution of LAT1 in predicting the prognosis of PC patients undergoing ADT. We are currently
planning a prospective study assessing expressional differences of LAT1 before and after treatment with MDV3100 and Abiraterone.

Conclusion

The current study found negative regulation of amino acid transporter LAT1 through AR. Increased expression of LAT1 under androgen deprivation therapy may potentially contribute to acquisition of castration resistance in PC cells. Since targeting only AR mediated pathway has limited efficacy especially after development of CRPC, dual inhibition of AR plus LAT1 may give us another opportunity to control CPRC progression. Future direction will be to study the association between LAT1 expression and acquisition of abiraterone and MDV3100 resistance in CPRC patients.

Acknowledgment

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References


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14. Wang Q, Bailey CG, Ng C et al. Androgen receptor and nutrient signaling pathways


Figure Legends

Figure 1. LAT1 expression in prostate cancer biopsy specimens

Representative immunohistochemical expressions of LAT1 in Gleason pattern 4 (black arrow head) and benign gland (white arrow head) are shown in low magnification (x20) (Figure A) and high magnification (x200) (Figure B). Gleason pattern 3 to 5 are shown in low magnification (x20) (Figure C) and Gleason pattern 3 (black arrow head) and Gleason pattern 4 (white arrow head) are shown in high magnification (x200) (Figure D).

Figure 2.

Kaplan-Meier analysis of PSA recurrence free survival related to LAT1 and AR expression in ADT patients. Figure A represents PSA recurrence free survival of prostate cancer patients underwent androgen deprivation therapy in LAT1 high and Low patients. Figure B represents PSA recurrence free survival of prostate cancer patients underwent androgen deprivation therapy in AR high and Low patients. Figure C represents PSA recurrence free survival of prostate cancer patients underwent androgen deprivation therapy stratified by LAT1 and AR expression. (*: P<0.05, **: P<0.01, ***: P<0.001)
Figure 3. Expression of LAT1 in prostate cancer cells

Protein (A) and m-RNA (B) levels of LAT1 were examined in prostate cancer cells, include LNCaP, C4-2, PC3, and DU145 cells. SiRNA of LAT1 (#1 and #2) significantly knocks down LAT1 expression in LNCaP (C and D) and C4-2 cells (E and F). Data are representative of three separate experiments. (**: P<0.01)

Figure 4. Functional significance of LAT1 in LNCaP and C4-2 cells

SiLAT1 inhibits cell proliferation (A, B), cell migration (C, D), and cell invasion (E, F) in LNCaP and C4-2 cells. Data are representative of three separate experiments. (*: P<0.05, **: P<0.01, ***: P<0.001)

Figure 5. Role of LAT1 in the m-TOR and AR-related pathways

Knock down of LAT1 protein inhibits phosphorylation of p70S6K and 4EBP-1, but not AKT (72 h) (A). SiAR increases LAT1 expression in LNCaP and C4-2 cells (protein level (B) and mRNA level (C)). DHT inhibits LAT1 expression, but adding anti-androgen (bicalutamide) increases LAT1 expression (D). Adding DHT to 5%-charcoal filtered FCS decreases LAT1 expression, while adding bicalutamide (10µM) or MDV3100 (10µM) reverses the effect of DHT (E). AR expression decreases
time dependently, while LAT1 increases time dependently with 5% charcoal filtered FCS (F). Data are representative of three separate experiments. (*: P<0.05, **: P<0.01)

Figure 6. Effect of dual inhibition of LAT1 and AR pathways in cell proliferation

Cell proliferation was examined in control, bicalutamide (10μM), SiLAT1, and SiLAT1+bicalutamide (10μM) at day 1 (24 h) to day 3 (72 h) in C4-2 cells (A) and LNCaP cells (B). Cell proliferation was also examined in control, MDV3100 (10 μM), SiLAT1, and SiLAT1+MDV3100 (10μM) at day 1 (24 h) to day 3 (72 h) in C4-2 cells (C) and LNCaP cells (D). Data are representative of three separate experiments. (*: P<0.05, **: P<0.01)

Supplementary figure 1.

Expression of LAT1 (A), AR (B) and PSA (C) in serial biopsy specimen.

PSA, along with AR and LAT1 expressions were higher in cancer lesion (black arrow head) compare to benign lesion (white arrow head).

Supplementary figure 2.

Expression of LAT1 (A), AR (B) and PSA (C) in serial biopsy specimen (Gleason 3 to
5). AR and LAT1 expressions were higher in Gleason 3 (black arrow head), 4 (white arrow head) and 5 lesion, however, PSA showed weak or no staining at same lesion.

Supplementary figure 3.

Negative correlation of AR and LAT1 staining in specific lesion.

LAT1 staining is low (B) in AR staining high lesion (A), while LAT1 staining is high (D) in AR staining low (C) lesion.

Supplementary figure 4.

LAT1 and cell proliferation

Overexpression of LAT1 in LNCaP cells showed only slight increase but no significant increase in cell proliferation (P=0.33) (A).

Knock down of LAT1 in DU145 (B) and PC3 (C) cells showed no remarkable difference in cell proliferation (P>0.05).
Key of Definitions for Abbreviations

ADT: Androgen deprivation therapy
CRPC: Castration resistant prostate cancer
PSA: Prostate specific antigen
LAT1: Human L-type amino acid transporter 1
AR: Androgen receptor
DHT: Dihydrotestosterone
TST: Testosterone
Table 1 Clinical factors associated with LAT1 expression in ADT patients

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BMI: body mass index (kg/m²); ALP: alkaline phosphatase (ng/dL); PSA: prostate specific antigen; PSAD: PSA density; TST: testosterone (ng/dL); TT nadir: time to nadir (month); cT stage: Clinical T stage; AR Score: Androgen receptor staining score.

Table 2 Risk of PSA recurrence in patients undergoing androgen deprivation therapy

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BMI: body mass index (kg/m²); cT stage: Clinical T stage; GS: gleason score; ALP: alkaline phosphatase (ng/dL); PSA: prostate specific antigen; LAT1: L-type amino acid transporter 1; AR: androgen receptor.
Figure 1

A

Cancer Lesion

B

Cancer Lesion (Gleason 4)

C

Benign gland

C

Gleason 3

D

Gleason 4
Figure 2

A. PSA recurrence free survival of ADT patients

B. PSA recurrence free survival of ADT patients

C. PSA recurrence free survival of ADT patients
Figure 3

A

LAT1

GAPDH

B

Relative mRNA expression (LAT1/GAPDH)

C4-2

PC-3

DU145

LAT1

GAPDH

nega siLAT1#1  siLAT1#2

C

LNCaP

Relative mRNA expression (LAT1/GAPDH)

nega  siLAT1#1  siLAT1#2

D

LAT1

GAPDH

E

C4-2

Relative mRNA expression (LAT1/GAP1DH)

nega  siLAT1#1  siLAT1#2

F

LAT1

GAPDH
Figure 4

A. LNCaP

B. C4-2

C. LNCaP

D. C4-2

E. LNCaP

F. C4-2
Figure 5

**A**

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Figure 6

**A** Bicalutamide 10 μM

- **Control**
- Bicalutamide 10 μM
- siLAT1 #1
- Bicalutamide 10 μM + siLAT1 #1

**B** LNCaP

- Control
- Bicalutamide 10 μM
- siLAT1 #1
- Bicalutamide 10 μM + siLAT1 #1

**C** MDV 3100 10 μM

- **Control**
- MDV 10 μM
- siLAT1 #1
- MDV 10 μM + siLAT1 #1

**D** LNCaP

- Control
- MDV 10 μM
- siLAT1 #1
- MDV 10 μM + siLAT1 #1
Supplementary figure 1

A  LAT1
  ▼ Cancer Lesion

▼ Cancer Lesion (Gleason 4)
▼ Benign gland

B  AR
  ▼ Cancer Lesion

▼ Cancer Lesion (Gleason 4)
▼ Benign gland

C  PSA
  ▼ Cancer Lesion

▼ Cancer Lesion (Gleason 4)
▼ Benign gland
Supplementary figure 2

A  LAT1

B  AR

C  PSA

▶ Gleason 3
▶ Gleason 4

▶ Gleason 3
▶ Gleason 4

▶ Gleason 3
▶ Gleason 4
Supplementary figure 3

A  AR High

B  LAT1 Low

C  AR Low

D  LAT1 High
Supplementary figure 4

LNCaP Cell LAT1 overexpression

A

![Graph showing relative cell growth (% of Day0) over days 0 to 4 for pcDNA3.1 and pcDNA3-hLAT1.](image)

DU145 and PC3 Cell siLAT1 Knock Down

B

![Graph showing relative cell growth (% of Day0) over days 0 to 4 for DU145 and PC3 with different siLAT1 knockdowns.](image)

C

![Graph showing relative cell growth (% of Day0) over days 0 to 4 for PC3 with different siLAT1 knockdowns.](image)