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Research article

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Transcriptomic data analysis of melanocytes and melanoma cell lines of LAT transporter genes for precise medicine

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Abstract: Background: Boron Neutron Capture Therapy (BNCT) is a two-step treatment that can be used in some types of cancers. It involves administering a compound containing boron atoms to the patient and irradiating the affected area of the body with a neutron beam. The success of the therapy depends mainly on the delivery of the boron isotope (^{10}B) to the tumor using an appropriate boron carrier. One of the boron carriers used is boronophenylalanine (BPA). Therefore, in research on the use of boron carriers, it is also important to know the mechanisms of its uptake by cells. Aim: To study the expression of LAT family genes in two melanoma (high melanotic WM115 and low melanotic WM266-4) cell lines and melanocytes (HEMa-Lp) which are responsible for the transport the BPA into cells. Methods: To normalize data from the transcriptomic analysis, the ratio of the median method was used. This allowed the samples to be compared with each other. Comparison metrics included log-fold change (LFC) values. The heatmap of LFC values and the cluster map were created. These graphs show the similarities and differences between the samples. Results: Transcriptomic data show that in melanocytes, LFC for SLC7A5 (LAT1) and SLC3A2 (4Fhc) was higher than in melanoma cell lines, which corresponded with their melanin content. Conclusion: Our results indicate overexpression of BPA transporter genes in normal cells (melanocytes), which may suggest the highest level of these proteins in melanocytes compared to less melanotic melanoma. Therefore, for BNCT, the use of BPA as the ^{10}B carrier will require additional qualifying tests of amino acid transporter expression for patients and specific tumors to develop a personalized BNCT.

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Introduction

Cell transport is one of the key processes in living organisms. Due to the exchange of compounds across the cell membrane, nutrients are delivered to a cell, waste metabolites and toxins are removed, as well as cell signaling, and drug delivery are possible. The proteins responsible for enabling the cellular exchange of these substances are transporters [1]. There are several dozen types of amino acids transporters, and one of the most important is the SLC family which includes SLC7A5 (LAT1), SLC7A8 (LAT2), SLC43A1 (LAT3), and SLC43A2 (LAT4). The abbreviation LATs means that their transport large neutral amino acids [2,3].

Large amino acid transporter 1 (LAT1) is a heterodimeric, sodium- and pH-independent amino acid transporter which has 12 transmembrane domains [4]. LAT1 is encoded by the SLC7A5 gene, which is located at 16q24.2 and consist of 39.5 kbp and 10 exons [5]. The highest expression of LAT1 is observed in the brain, spleen, placenta, testis, bone marrow, colon, and fetal liver [4,5]. LAT1 is formed with 4F2hc complex LAT1/CD98 which is responsible for membrane localization and proper transporter function [1]. LAT1 imports essential amino acids such as methionine, histidine, leucine, tryptophan, phenylalanine, and tyrosine in exchange for intracellular amino acids such as glutamine. It also plays a role in the transport of L-DOPA across the blood-brain barrier (BBB) and thyroid hormones (T3 and T4) across the cell membrane. LAT1 is involved in growth, proliferation, cell signaling and tissue development [5]. LAT1 overexpression is observed in many tumors such as non-small cell lung cancer, biliary duct cancer, pancreatic cancer, and prostate cancer, and the poor prognosis of luminal B type of breast cancer [1,6,7]. Therefore, LAT1 is proposed as a new target for tumor-specific drug delivery and anticancer therapy [3,8,9]. LAT1 is also highly expressed in skin cancer and has been suggested as a biomarker for malignant melanoma progression [10]. On the other hand, decreased expression of LAT1 is observed in the BBB in Parkinson's disease, and mutations changing LAT1 functionality are described in Autism Spectrum Disorders [5,11].

The SLC7A8 gene encoding LAT2 is located at 14q11.2 and like LAT1, LAT2 is associated with the glycoprotein 4F2hc, and their sequences are in 50% homogenous [4]. LAT2 is mainly expressed in the kidney, jejunum, ileum, lung, heart, and spleen [12]. In contrast to LAT1, LAT2 transports thyroid hormones only inwards and the transport of the drug via BBB is negligible [13]. LAT2 transports small and large neutral amino acids and has a higher affinity for L-phenylalanine than LAT1. Its function is the reabsorption of neutral amino acids from the epithelial cells to the bloodstream in the kidneys and the homeostasis of ion metals [14]. LAT3 and LAT4 are expressed in the pancreas, liver, and skeletal muscle. They have a high affinity to transport large neutral amino acids. LAT3 is involved in the development of prostate cancer in humans [4,15].

Boron Neutron Capture Therapy (BNCT) is a two-step procedure, used when other treatments are ineffective or not applicable. This therapy may be promising for malignant melanoma of the skin, which is usually detected at the advanced stage. BNCT therapy consists in administering a compound containing the ^{10}B isotope into the patient's body, followed by irradiation with a beam of thermal neutrons. The increased metabolism of the cancer cells resulted in a higher concentration of ^{10}B in them than in surrounding normal cells. During neutron radiation, due to the large cross-section and due to the high concentration of ^{10}B, a neutron capture nuclear reaction takes place. The products of which (helium and lithium nuclei) have high energy and short range (in terms of cell diameter). The result of this reaction is damage to only cancer cells, whereas normal ones stay almost intact [16,17]. Despite the many advantages of BNCT therapy, there are also some serious disadvantages as the availability of neutrons sources, the determination of thermal neutron doses, and boron carriers that must not only bring tumor cells to the appropriate concentration of boron, but also not be uptaken by normal, healthy cells. Only two boron carriers are used: BSH (sodium borocaptate, $Na_2B_{12}H_{11}SH$) and BPA (p-boronophenylalanine, $C_9H_{12}BNO_4$). They differ in cellular uptake, BSH enters the cell by diffusion, unlike BPA which is actively taken up by cells through membrane proteins. However, they do have their drawbacks such as the non-uniform distribution of ^{10}B in the cell [18] and diverse sensitivity to neutron irradiation of human melanoma cells [19]. Therefore, the greatest advantage of the therapy, which is the selective destruction of cancer cells, depends on the delivery of right amount of the boron isotope ^{10}B to the cells. For this reason, the research on the boron carrier and its effective delivery is still being intensively carried out to increase and ensure high efficiency of the therapy. The large neutral amino acid transporter (LAT1) is considered the most likely candidate for BPA transport to the cell [20], but its expression in cancer cells is scarcely known.

The aim of this study was to investigate the expression of LAT family genes in two melanoma cell lines: primary WM115 and metastatic WM266-4 and compare this expression with human healthy melanocytes HEMa-Lp. This study was preliminary to further research on LAT protein expression and activity, and BPA uptake in different melanotic cell lines.

Methods

Data preparation

Raw transcriptomic data (represented as count values) is stored at the Jagiellonian University Repository (RUJ) [21].

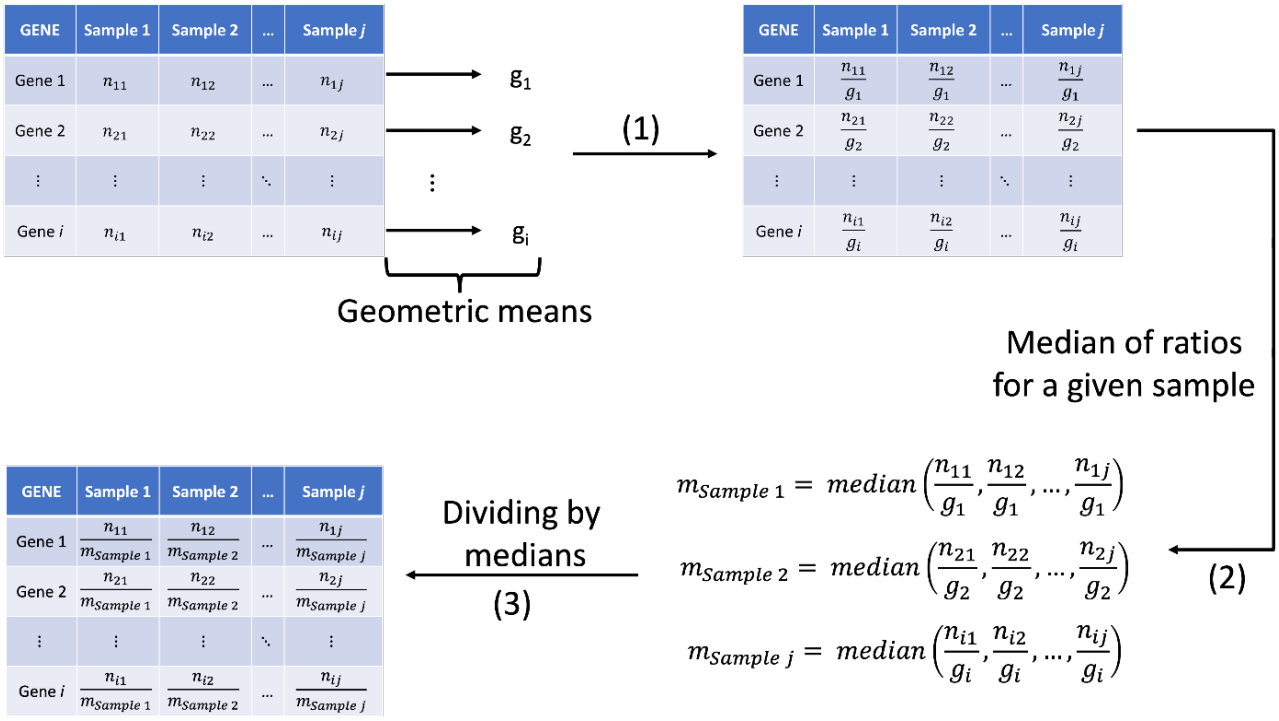

Figure 1: Schematic way of data normalization procedure. (1) Dividing by the geometric mean calculated for each gene, (2) Determining median for each sample, (3) Dividing raw counts of each sample by its appropriate median value. Such process results dataset, which is ready to be used in DGE analysis.

Fastq files containing paired reads from transcriptome sequencing of 9 human cell line samples WM115, WM226-4, and HEMa-Lp (in triplicates of each experimental groups were analyzed). The assessment of the quality of the readings was carried out with the FastQC tool (version 0.11.8) and did not rise any objections. After quality assessment, the raw reads were mapped to the human GRCh38 reference genome from the Ensembl database (version 96) using Hisat2 software (version 2.1.0). The Cuffquant and Cuffmerge programs (version 2.2.1) and the GTF file Homo_sapiens.GRCh38.96.gtf from the Ensembl database were used [21]. In addition, only the correct biotypes (encoding protein) were retained and genes with the mean of counts above the threshold (equal to 10) were analyzed. The data prepared in this a way can be later subjected to normalization and differential gene expression (DGE) analysis.

Data normalization and analysis

Data normalization was done according to the Median Ratio Normalization (MRN), which is used i.e., in the DESeq package, within R (Bioconductor) software [22]. This normalization method finds the geometric mean for each gene in all samples, takes median of these values (size factor), and then divides the counts by the values of the size factors (schematic way of data normalization was shown in the Fig. 1). Such method was proved to be more robust in comparison to the other normalization methods and allows to compare the global differences between the samples, which is crucial for the DGE analysis [23,24]. The normalization, visualizations and metrics calculations were performed in Python (version 3.8.1) using numpy v. 1.20.1, seaborn v. 0.11.1, matplotlib v. 3.3.4, pandas v. 1.2.4 and scikit-learn v. 0.24.1.

DGE analysis was done for the following comparisons HEMa-Lp vs. WM115, HEMa-Lp vs. WM266-4, and WM115 vs. WM266-4. In comparisons between normal and cancerous cell lines HEMa-Lp was treated as a reference (control) sample. The first step in the DGE analysis was defining the parameters used in the comparison between the samples. Such parameters include log-fold change (LFC), which is defined as the ratio between the counts of abnormal (pathological) and normal (healthy) cells. LFC values can be described with any real value. Conventionally, thresholds for LFC are set for values greater than the absolute value of 2. Additionally, z statistics was calculated, defined as the ratio between the LFC value and the standard deviation of LFC within a given group. Based on this values Wald p-value was calculated, along with the adjusted p-values.

Adjusted p-values are calculated according to

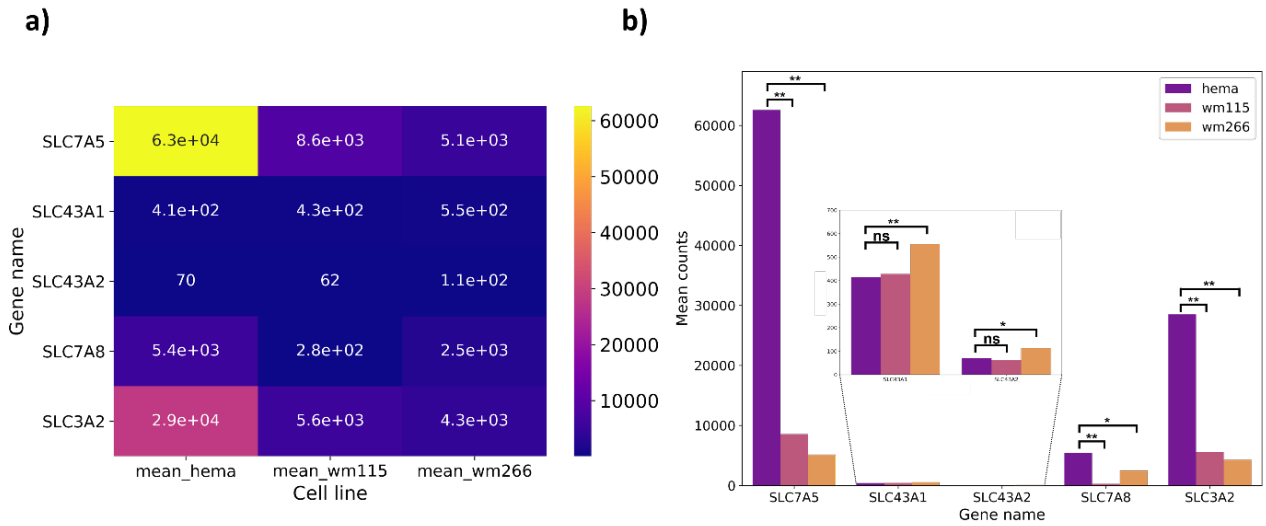

Figure 2: The expression level of genes SLC7A5 (LAT1), SLC43A1 (LAT3), SLC43A2 (LAT4), SLC7A8 (LAT2), and SLC3A2 (4F2hc) for melanocytes (hema) and melanoma cell lines: WM115 and WM266-4. a) Heatmap showing normalized expression levels of analyzed genes. Different colors represent values of counts, according to the color bar. b) Bar plot showing statistical differences (FDR p-values, calculated as described in Methods section) between cell lines' gene expressions, * $p < 0.05$, ** $p <$ 1e-6, ns – not significant.

the Benjamini-Hochberg procedure (allowing for reducing the false discovery rate, FDR) [25]. The correction for FDR is crucial, because the transcriptomics dataset is large (thousands of genes), and thus prone to the type I errors.

Results/Discussion

The transcriptomic data and the method used to allow for the analysis of differences in the expression of LAT genes between the tested cell lines, i.e., HEMa-Lp, WM115, and WM266-4. HEMa-Lp is a cell line derived from donor normal skin melanocytes, while WM115 and WM266-4 are melanoma cell lines from one patient [26]. They differ in origin and melanin content. WM115 is derived from a primary tumor and contains melanin, but in lower concentration than melanocytes, while WM266-4 is metastatic and depigmented [27]. Figure 2 shows comparisons of gene expression level (as a normalized counts number) for normal (*hema*) and two melanomas (*wm115* and *wm266-4*). Statistical differences were observed between mean expression levels of analyzed genes between normal and cancerous cell lines (Fig.2b). These results showed that the highest expression of the SLC7A5 (LAT1) gene was found in the normal cell line (HEMa-Lp). For this cell line, the second highest level of expression was observed for the SLC3A2 gene (Fig. 2a). This is because this gene encodes a heavy chain (4Fhc) subunit necessary for the proper function of LAT1 [10]. This relationship was also evident for WM115 and WM266-4 where we observed the highest expression levels of the SLC7A5 (LAT1) and SLC3A2 (4Fhc) genes. In addition, the lowest level of expression of the SLC43A2 (LAT4) gene was common to all cell lines. The SLC7A8 (LAT2) gene was also highly expressed in normal cells (HEMa-Lp). In WM266-4 this gene has two-fold lower expression level, while in WM115 expression was significantly lower in comparison to normal cells (Fig. 2b).

In our study, we observed a high levels of the LAT2 gene in melanocytes, which is characteristic for normal tissues. Surprisingly, normal cells had the highest levels of the expression of the LAT1 gene. LAT1, which was recognized as a tumor characteristic and proposed as a prognostic marker for cancer processes has the expression in the normal cell line that exceeds metastatic cell line almost 16 times. Also, the primary melanoma cell line (WM115) has higher expression than the metastatic cell line – approximately 2 times.

Figure 3 shows the logarithmic change (LFC) values for each LAT gene family for each tested cell line compared to each other. In this graph, one can see which genes were overexpressed and which were underexpressed and magnitude of this over/underexpression. In addition, this chart allows us to group individual comparisons and find out which ones were most similar to each other. For the SLC43A2 gene LFC values indicate almost 2-fold overexpression in

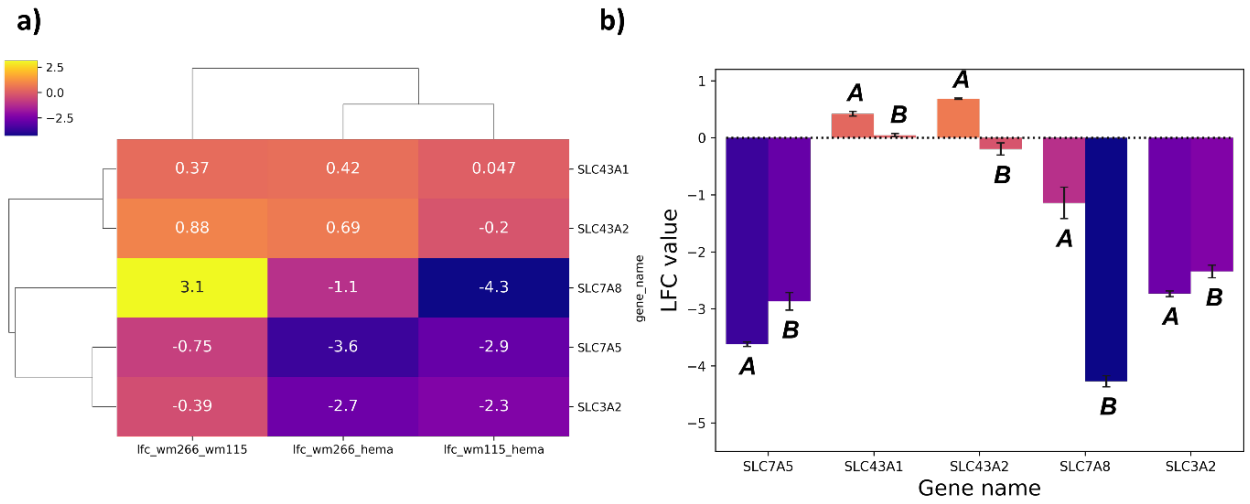

Figure 3: Log-fold change values for each cell line for LAT family genes. a) Heatmap showing LFC values (numbers of the graph) as a comparison of each cell line to each other. Colors on the graph correspond to the color bar in the left-hand corner. b) LFC values shown as bars representing under- or overexpression of each gene in melanoma cell lines compared to HEMa-Lp (error bars calculated as a standard deviation of LFC). A, B indicate LFC values for WM266-4 and WM115 cell line, respectively.

WM266-4 vs. WM115 cell line. In the case of WM266-4 vs. HEMa-Lp, LFC values for both SLC43A1 and SLC43A2 are on a similar level and are the only ones with expression level, which is higher in melanoma lines than in melanocytes (Fig. 3b). Generally, expression levels of SLC43A1 and SLC43A2 were on the same low levels for all three cell lines. On the other hand, the expression of SLC7A5 and SLC3A2 was highest in normal then primary and the lowest in metastatic cell lines. Moreover, LAT gene expression levels were more similar between melanoma cell lines than between melanocytes and any melanoma cell line. However, the expression level of SLC7A8 (LAT2) was 8.6 times higher in WM266-4 than WM115 (Fig. 3a). The LAT2 expression levels are lower in metastatic than normal cell lines. When comparing the primary melanoma with the normal melanocytes, the highest difference is in the SLC7A8 (LAT2) genes, which was over 16-fold smaller in the case of WM115. Therefore, the expression level of SLC7A8 is the higher in a normal cell line, then in the metastatic and the lowest in primary cell line.

(^{10}B)-BPA enhances cell damage during neutron irradiation only for the melanotic cells. Less melanotic melanoma was less sensitive to the thermal neutron beam probably because of insufficient uptake of ^{10}B by these cells [26]. In our study, we observed that less melanotic cells (WM266-4) had lower expression of the LAT1 gene, which supports these observations.

Conclusion

Our transcriptomic studies for two melanoma cell lines (WM115 and WM266-4) and melanocytes (HEMa-Lp) showed how important is to characterize the transport and metabolism of amino acids in cells that are potential therapeutic targets for therapies based on phenylalanine derivatives (BPA). Literature data suggest the highest level of expression of the LAT1 gene in cancer cells [19], thus LAT1 may serve as a diagnostic and prognostic marker for phenylalanine metabolizing cells [3,10]. However, as for SLC7A8 (LAT2), the gene expression was higher in the WM266-4 than in WM115 cell line, the expression of LAT2 in melanoma cancer is not prognostic [28,29]. Our data showed that the melanocytes (normal cells) have the highest levels of expression of SLC7A5 gene as well as the SLC7A8 gene, which may influence the ^{10}B uptake and accumulation. Whereas, for melanoma cell lines, the expression of the SLC7A5 gene in WM115 (primary tumor cell line) is higher than in WM266-4 (malignant cell line), in contrast to the expression of the SLC7A8 gene, which is lower in WM115 than in WM266-4.These results suggest that in the case of BNCT the use of BPA as a ^{10}B carrier will require additional qualifying tests (e.g. BPA molecular transporters) in patients before BNCT, especially for the treatment of less melanotic cancer [19]. To confirm these results, proteomic and functional tests are needed in further investigations.

References

[1] Kanai Y. Amino acid transporter LAT1 (SLC7A5) as a molecular target for cancer diagnosis and therapeutics. Pharmacol Ther. 2022;230:107964.

[2] Alles SRA, Gomez K, Moutal A, Khanna R. Putative roles of SLC7A5 (LAT1) transporter in pain. Neurobiology of Pain. 2020;8:100050.

[3] Achmad A, Lestari S, Holik HA, Rahayu D, Bashari MH, Faried A, et al. Highly specific l-type amino acid transporter 1 inhibition by JPH203 as a potential pan-cancer treatment. Processes. 2021;9:1–15.

[4] Wang Q, Holst J. L-type amino acid transport and cancer: Targeting the mTORC1 pathway to inhibit neoplasia. Am J Cancer Res. 2015;5:1281–94.

[5] Scalise M, Galluccio M, Console L, Pochini L, Indiveri C. The human SLC7A5 (LAT1): The intriguing histidine/large neutral amino acid transporter and its relevance to human health. Front Chem. 2018;6:1–12.

[6] Higuchi K, Sakamoto S, Ando K, Maimaiti M, Takeshita N, Okunushi K, et al. Characterization of the expression of LAT1 as a prognostic indicator and a therapeutic target in renal cell carcinoma. Sci Rep. 2019;9:1–10.

[7] Liu YH, Li YL, Shen HT, Chien PJ, Sheu GT, Wang BY, et al. L-type amino acid transporter 1 regulates cancer stemness and the expression of programmed cell death 1 ligand 1 in lung cancer cells. Int J Mol Sci. 2021;22:10955 .

[8] Dickens D, Chiduza GN, Wright GSA, Pirmohamed M, Antonyuk S v., Hasnain SS. Modulation of LAT1 (SLC7A5) transporter activity and stability by membrane cholesterol. Sci Rep. 2017;7:1–13.

[9] Detta A, Cruickshank GS. L-Amino Acid Transporter-1 and Boronophenylalanine-Based Boron Neutron Capture Therapy of Human Brain Tumors. Cancer Res. 2009;69:2126–32.

[10] Shimizu A, Kaira K, Kato M, Yasuda M, Takahashi A, Tominaga H, et al. Prognostic significance of L-type amino acid transporter 1 (LAT1) expression in cutaneous melanoma. Melanoma Res. 2015;25(5):399–405.

[11] Cascio L, Chen CF, Pauly R, Srikanth S, Jones K, Skinner CD, et al. Abnormalities in the genes that encode Large Amino Acid Transporters increase the risk of Autism Spectrum Disorder. Mol Genet Genomic Med. 2020;8:1–12.

[12] Scala I, Concolino D, Nastasi A, Esposito G, Crisci D, Sestito S, et al. Beneficial effects of slow-release large neutral amino acids after a phenylalanine oral load in patients with phenylketonuria. Nutrients. 2021;13:4012.

[13] Krause G, Hinz KM. Thyroid hormone transport across L-type amino acid transporters: What can molecular modelling tell us? Mol Cell Endocrinol. 2017 Dec 15;458:68–75.

[14] SLC7A8 - Large neutral amino acids transporter small subunit 2 - Homo sapiens (Human) | UniProtKB | UniProt

[15] SLC43A1 - Large neutral amino acids transporter small subunit 3 - Homo sapiens (Human) | UniProtKB | UniProt

[16] Nedunchezhian K, Aswath N, Thiruppathy M, Thirugnanamurthy S. Boron neutron capture therapy - a literature review. Journal of Clinical and Diagnostic Research. 2016;10:ZE01–4.

[17] Silarski M, Dziedzic-Kocurek K, Szczepanek M. Combined BNCT and PET for theranostics. Bio-Algorithms and Med-Systems. 2021;17:293–300.

[18] Dymova MA, Taskaev SY, Richter VA, Kuligina EV. Boron neutron capture therapy: Current status and future perspectives. Cancer Commun. 2020;40:406–21.

[19] Brown K, Mountford MH, Allen BJ, Mishima Y, Ichihashi M, Parsons P. Neutron Irradiation of Human Melanoma Cells. Pigment Cell Res. 1989;2(4):319–24.

[20] Wongthai P, Hagiwara K, Miyoshi Y, Wiriyasermkul P, Wei L, Ohgaki R, et al. Boronophenylalanine, a boron delivery agent for boron neutron capture therapy, is transported by ATB0,+, LAT1 and LAT2. Cancer Sci. 2015;106:279–86.

[21] Kubicz E, Stępień E. Results Transcriptomes Sequencing Dataset for Human Melanoma and Melanocytes Cell Cultures. Kraków : Repozytorium Uniwersytetu Jagiellońskiego, 2021. https://doi.org/10.26106/y8e9-7603.

[22] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):1–21.

[23] Maza E, Frasse P, Senin P, Bouzayen M, Zouine M. Comparison of normalization methods for differential gene expression analysis in RNA-Seq experiments: A matter of relative size of studied transcriptomes. Commun Integr Biol. 2013;6(6).

[24] Evans C, Hardin J, Stoebel DM. Selecting between-sample RNA-Seq normalization methods from the perspective of their assumptions. Brief Bioinform. 2018;19(5):776–92.

[25] Chen SY, Feng Z, Yi X. A general introduction to adjustment for multiple comparisons. J Thorac Dis. 2017;9(6):1725–9.

[26] Herlyn M, Balaban G, Bennicelli J, Guerry IV D, Halaban R, Elder DE, et al. Primary melanoma cells of the vertical growth phase: Similarities to metastatic cells. J Natl Cancer Inst. 1985;74:283-9.

[27] Kubicz E. Biomedical applications of Positron Annihilation Lifetime Spectroscopy: nanostructural characterization of normal and cancer cells and tissues [Ph.D. thesis]. Kraków, Jagiellonian University; 2020.

[28] Uhlen M, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhori G, et al. A pathology atlas of the human cancer transcriptome. Science. 2017;357:eaan2507.

[29] Expression of LAT2 in melanoma - The Human Protein Atlas. Available from: https://www.proteinatlas.org/ENSG00000086730-LAT2/pathology/melanoma