Cumhuriyet Sci. J., 46(3) (2025) 506-511
DOI: https://doi.org/10.17776/csj.1698280

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Metabolic Adaptations: Comparison of Amino Acid Profiles of SK-MEL-30 Melanoma Cells and CCD-1135Sk Fibroblasts

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Research Article

History
Received: 13/05/2025
Accepted: 18/09/2025

ABSTRACT

Melanoma is the most aggressive form of skin cancer, characterized by high metastatic potential and poor prognosis. Tumor cells undergo metabolic reprogramming to support rapid proliferation, survival, and invasion. In this study, we provide original data on the metabolic adaptations of melanoma cells by comparing amino acid profiles between SKMEL-30 melanoma cells and healthy human dermal fibroblasts (CCD-1135Sk). Quantitative amino acid analyses using LC-MS/MS revealed that the total amino acid load in melanoma cells was approximately 80% higher than in fibroblasts (7595.94 ng/mL vs. 4225.35 ng/mL). Significant elevations were observed in glutamate, valine, leucine, isoleucine, serine, alanine, threonine, and glycine, reflecting increased energy demand, redox maintenance, biosynthesis, and stress response. Additionally, taurine and GABA levels were markedly increased, suggesting that melanoma cells acquire enhanced survival capabilities under stress conditions. These findings highlight the distinctive amino acid metabolism of melanoma and underscore its potential for the development of novel biomarkers and targeted metabolic therapies.

Keywords: Melanoma, Amino acid metabolism, LC-MS/MS, Cell proliferation.

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Introduction

Cancer is a complex disease characterized by uncontrolled cellular growth and proliferation, driven by various genetic and metabolic alterations. Recent studies have highlighted that cancer cells undergo metabolic reprogramming to adapt to environmental stressors, leading to profound changes in energy production, amino acid metabolism, and the synthesis of cellular components [1,2]. Efficient utilization of glucose, amino acids, and other nutrients is critical for supporting rapid proliferation, and metabolic flexibility is a key strategy that enables tumor survival, growth, and metastasis [2,3].

Melanoma, a malignancy originating from skin melanocytes, is particularly aggressive and capable of rapid proliferation and metastasis. Melanoma cells, including SK-MEL-30, exhibit high metabolic plasticity to meet their energetic and biosynthetic demands, allowing them to adapt to nutrient fluctuations and environmental stress [3,4]. These metabolic adaptations extend beyond energy production, influencing essential biological processes such as protein synthesis, cellular structure maintenance, and antigen presentation [4,5].

Fibroblasts, as connective tissue cells responsible for producing collagen and extracellular matrix components, normally display limited metabolic flexibility. However, in the tumor microenvironment, fibroblasts can undergo metabolic changes through interactions with cancer cells and various signaling pathways [5]. Such adaptations may support tumor progression by supplying essential metabolites or altering nutrient availability, highlighting the importance of studying stromal–tumor metabolic interplay.

Amino acids play pivotal roles in energy generation, protein synthesis, and the formation of other biomolecules. Rapidly proliferating cancer cells, such as melanoma cells, intensively consume amino acids, utilizing them as part of their metabolic reprogramming to sustain growth and survival [6,7]. Consequently, the amino acid profile of SK-MEL-30 melanoma cells is expected to differ substantially from that of normal fibroblasts, reflecting cancer-specific metabolic adaptations.

The present study aims to systematically compare the amino acid profiles of SK-MEL-30 melanoma cells and CCD-1135Sk fibroblasts using LC-MS/MS analysis. This comparison seeks to elucidate the metabolic differences between malignant and normal cells, identify potential metabolic vulnerabilities, and provide insights that may inform the development of novel therapeutic strategies targeting amino acid metabolism in melanoma.

Materials and Methods

Cell Culture

SK-MEL-30 melanoma cells were obtained from the Ankara ŞAP Institute Collection. Cells were cultured in a

DMEM:Hams F-12 mixture (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; HyClone) and 1% penicillin-streptomycin (Gibco, Carlsbad, CA, USA) at 37 °C under 5% CO_2. CCD-1135Sk fibroblast cells were obtained from the same source and cultured in DMEM (HyClone) with the same supplements and conditions. All experiments were performed using three biological replicates, and each sample was analyzed in three technical replicates.

Amino Acid Profiling with LC-MS/MS

After the incubation period, cells were detached using trypsin and centrifuged at 2500 g to separate the supernatant. The resulting pellets were resuspended in 5 mL of extraction solvent (1% formic acid in ultrapure water) and subjected to ultrasonic bath treatment at 50 °C for 10 minutes. Following centrifugation, 50 µL of each sample was transferred into Eppendorf tubes and mixed with 50 µL of internal standard (ISTD, provided in the Jasem Quantitative Amino Acid LC-MS/MS Analysis Kit) and 700 µL of amino acid (AA) reagent. The AA reagent was prepared according to the manufacturer's instructions using 5 mL of mobile phase B and 1 mL of mobile phase A. The mixtures were centrifuged at 1350 rpm for 5 minutes, and the clear supernatants were transferred into glass vials for analysis. All samples were analyzed in triplicate. Chromatographic separation was performed on a Zorbax HILIC column (3.5 µm, 4.6 mm) using an Agilent 1260 Infinity LC system coupled to an Agilent 6460 Triple Quadrupole MS/MS equipped with a Jet Stream Electrospray Ionization source. The mobile phases consisted of (A) 3% formic acid, ammonium formate (10 mM), and 50 mL methanol in ultrapure water, and (B) acetonitrile. The flow rate was set at 0.7 mL/min with a column temperature of 30 °C. The injection volume was optimized according to kit recommendations. The ion source parameters were as follows: gas temperature 150 °C, gas flow 10 L/min, and nebulizer pressure 40 psi.

Statistical Analyses

Data are expressed as mean ± standard deviation (SD). Differences between groups were analyzed using an unpaired t-test, with statistical significance accepted at $p < 0.05$.

Principal Component Analysis (PCA)

PCA was performed to visualize similarities and differences in amino acid profiles between SK-MEL-30 melanoma cells and CCD-1135Sk fibroblasts. The analysis was conducted using PAST software. The first two principal components (PC1 and PC2) explaining the highest variance were plotted, and loadings were calculated to identify amino acids contributing most to group separation. PCA enabled the identification of key metabolites distinguishing melanoma cells from fibroblasts and provided insights into metabolic adaptations.

Results

In this study, the amino acid profiles of SKMEL-30 (melanoma) and CCD-1135Sk (normal fibroblast) cells were compared in detail. Three biological replicates and three technical replicates were analyzed for each cell line. Significant differences in amino acid metabolism were observed in melanoma cells, reflecting their high proliferative and metastatic potential. The total amino acid concentration was approximately 80% higher in SKMEL-30 cells (7595.94 ng/mL) compared to CCD-1135Sk cells (4225.35 ng/mL). When expressed as fold change (SKMEL-30 / CCD-1135Sk), key amino acids showed the following increases: glutamate 2.2-fold, aspartate 1.8-fold, glycine 1.6-fold, taurine 2.0-fold, and GABA 1.7-fold. Significant increases were also observed in BCAA group amino acids: leucine (1.5-fold), isoleucine (1.4-fold), and valine (1.6-fold), supporting the role of BCAA metabolism via mTOR pathways in sustaining melanoma cell proliferation. Additionally, metabolite derivatives such as phospho-serine (1.4-fold) and carnosine (1.3-fold) were significantly elevated in SKMEL-30 cells, indicating enhanced stress response and membrane integrity maintenance. In CCD-1135Sk cells, only serine (1.1-fold) and threonine (1.1-fold) levels were slightly elevated but these differences were of limited biological significance. Figure 1 presents the amino acid concentrations, with fold changes and statistical significance (Student's t-test, $p < 0.05$) indicated for each metabolite. The pronounced elevation in glutamate highlights the dependence of melanoma cells on glutaminolysis, while increased BCAA levels emphasize their contribution to growth and proliferation. Preliminary principal component analysis (PCA) using Past software confirmed a distinct clustering of SKMEL-30 and CCD-1135Sk samples based on their amino acid profiles. Since only two samples were analyzed, PC1 explained 100% of the total variance. The PCA plot (Figure 2) demonstrates clear separation between the two cell lines, and loadings analysis identified glutamate, leucine, and taurine as the primary contributors to this separation. The PCA plot (Figure 2) demonstrates clear separation between SKMEL-30 and CCD-1135Sk cells, and loadings analysis identified glutamate, leucine, and taurine as the primary contributors to this separation.

Overall, these findings reveal hyperactive amino acid metabolism in melanoma cells, consistent with metabolic reprogramming that supports their high proliferation and invasive potential.

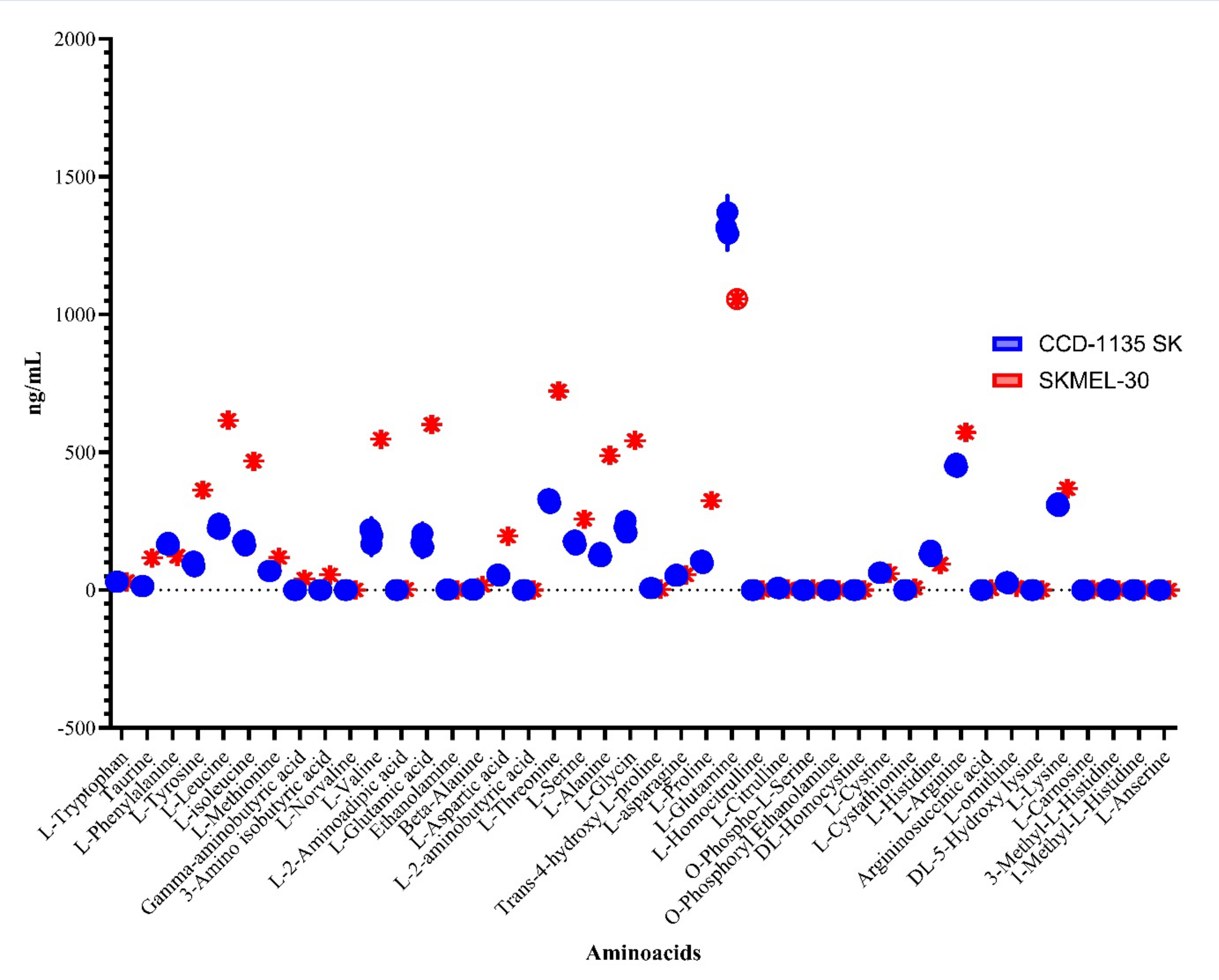

Figure 1. Amino Acid Concentrations Determined by LC-MS/MS in SKMEL-30 and CCD-1135Sk Cell Lines

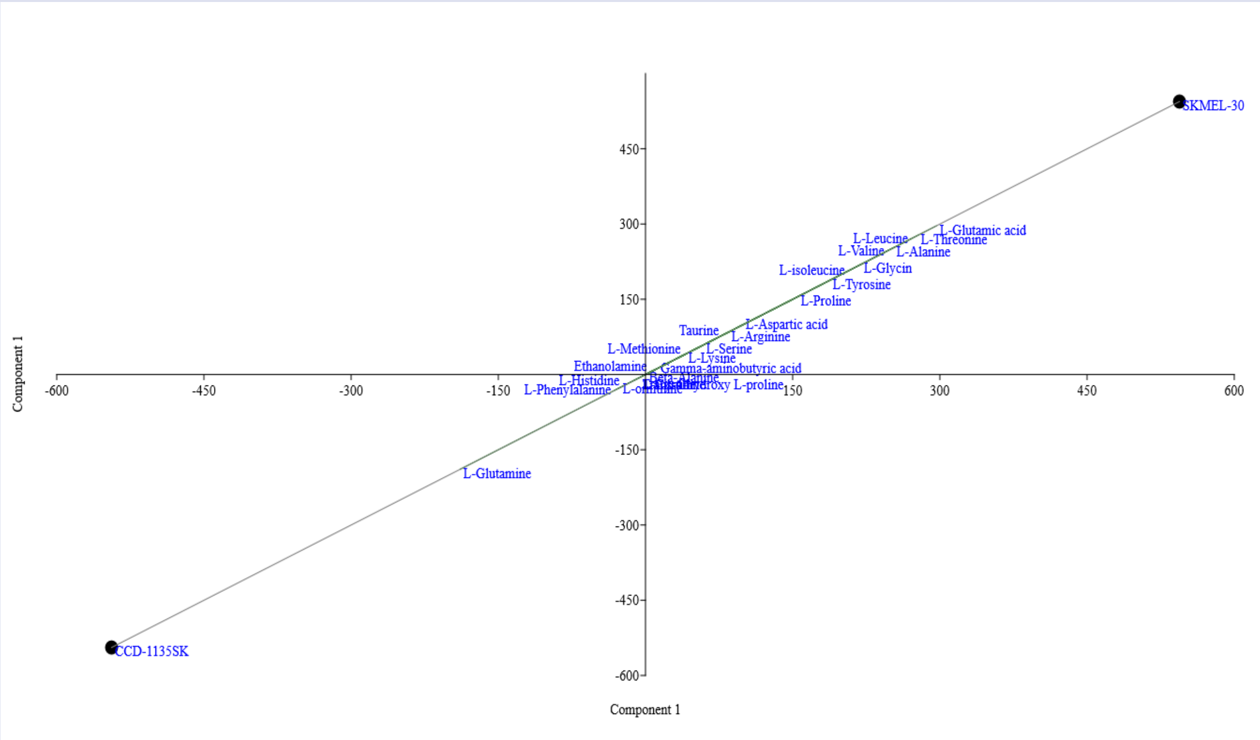

Figure 2. PCA plot of amino acid profiles in SKMEL-30 melanoma cells and CCD-1135Sk fibroblasts.

Discussion

Melanoma is the most lethal skin cancer due to its high malignant potential and metastatic capacity. Originating from melanocytes, this tumor induces dramatic metabolic alterations, reprogramming biochemical pathways that sustain survival, proliferation, and invasion [6]. Recent studies have shown that cancer cells remodel not only glucose but also amino acid metabolism to meet their increased biosynthetic and energetic demands [7].

In our study, a significant increase in multiple amino acids was observed in melanoma cells compared to normal fibroblasts. Elevated levels of branched-chain amino acids (BCAAs: leucine, isoleucine, valine) are particularly noteworthy, as these metabolites are well known to promote tumor progression through activation of mTOR signaling pathways [9]. High concentrations of serine and threonine further contribute to protein synthesis and epigenetic regulation via the one-carbon and methylation cycles [13]. Serine can also be converted to phosphoserine, participating in signaling pathways that support proliferation and resistance to apoptosis.

Alterations in other amino acids highlight the metabolic adaptability of melanoma. Elevated proline and glycine levels may facilitate extracellular matrix remodeling, with proline contributing to collagen biosynthesis and energy regulation, while glycine participates in both the folate cycle and gluconeogenesis [14,17]. Increased arginine levels point to enhanced nitric oxide and polyamine synthesis, processes linked to DNA synthesis, signaling, and the establishment of an immunosuppressive microenvironment [15]. Changes in cystine, methionine, and sulfur-containing intermediates (e.g., cystathionine, homocystine) indicate that the xCT antiporter system is activated, promoting glutathione synthesis and protection against oxidative stress [16]. Taurine accumulation suggests additional reinforcement of osmoregulation, membrane stability, and antioxidant defense, whereas elevated gamma-aminobutyric acid (GABA) may contribute to proliferative and migratory signaling [11,21].

Glutamine metabolism represents another crucial axis. While CCD-1135Sk fibroblasts displayed relatively high glutamine levels, likely acting as nutrient suppliers in the tumor microenvironment, melanoma cells showed marked glutamate enrichment. This reflects enhanced glutaminolysis, which sustains mitochondrial energy production, nucleotide biosynthesis, and redox homeostasis through glutathione synthesis [3,10,18–20]. Such glutamine dependency is a well-recognized metabolic vulnerability of melanoma and underscores its relevance for therapeutic targeting [22].

PCA analysis confirmed a distinct clustering of melanoma and fibroblast samples, with BCAAs and glutamine contributing most strongly to this separation. This finding supports the notion that amino acid metabolism is a defining feature of melanoma cells, distinguishing them from normal fibroblasts and highlighting potential biomarkers for disease characterization. The distinct amino acid signatures identified in melanoma cells are consistent with previous metabolomics studies in other tumor types. For example, Zhao et al. (2016) reported upregulated serine and glycine metabolism in colon cancer, while Soga et al. (2011) demonstrated increased BCAAs in pancreatic cancer [23,24]. Our findings support these observations and extend them to melanoma, revealing potential amino acid biomarkers and therapeutic targets.

Collectively, these results demonstrate that melanoma cells exploit multiple amino acid metabolic pathways, enabling metabolic flexibility beyond the classical Warburg effect. In contrast, healthy fibroblasts maintain lower and more balanced amino acid levels, reflecting reduced biosynthetic and energetic demands. The activation of atypical pathways, such as those involving 3-aminobutyric acid and argininosuccinic acid detected only in melanoma cells, further highlights the metabolic reprogramming characteristic of this malignancy. These findings highlight potential interventions, such as targeting BCAA transporters or glutamine metabolism, which are already under investigation in melanoma therapy. Such strategies may offer novel therapeutic avenues to selectively disrupt the metabolic dependencies of melanoma cells while sparing normal tissues.

A quantitative comparison of amino acid profiles between melanoma cells (SK-MEL-30) and normal dermal fibroblasts (CCD-1135Sk) using LC-MS/MS revealed substantial metabolic reprogramming in melanoma cells, with an overall ~80% increase in total amino acid load. Elevated levels of glutamate, branched-chain amino acids (valine, leucine, isoleucine), serine, and glycine directly support proliferation, energy production, redox balance, and stress adaptation, highlighting their role in tumor biology. PCA analysis confirmed clear separation between melanoma and fibroblast samples, suggesting that these amino acid signatures may serve as reliable biomarkers. These metabolic differences also indicate potential therapeutic strategies, such as targeting BCAA transporters or glutamine metabolism. However, the study has limitations, including its in vitro design, the use of a single melanoma cell line, and the absence of in vivo validation, which should be addressed in future studies to confirm the generalizability and translational relevance of these findings.

Conclusion

This study revealed significant differences in amino acid metabolism between melanoma cells and healthy dermal fibroblast cells. Amino acid levels were generally elevated in the SKMEL-30 cell line, with particularly high concentrations of metabolites such as glutamate, valine, leucine, serine, and taurine. These differences are directly associated with the increased proliferation capacity, energy production, redox balance, and stress adaptation of melanoma cells. In contrast, the low amino acid levels observed in normal fibroblasts reflect a balanced metabolism and limited biosynthetic demands. PCA analysis confirmed a clear separation between melanoma and fibroblast samples, supporting that amino acid signatures may serve as reliable biomarkers. These findings underscore that melanoma cells undergo comprehensive reprogramming of amino acid metabolism, which is directly linked to the aggressive biological behavior of the tumor. Future studies should evaluate inhibitors of BCAA transporters and glutamine metabolism in in vivo melanoma models, and assess whether similar metabolic patterns exist across different melanoma subtypes. Additionally, the potential of combining amino acid-targeted interventions with existing immunotherapies or targeted therapies warrants investigation in a multidisciplinary approach. Overall, these data contribute to a deeper understanding of melanoma metabolism and highlight amino acid pathways as promising candidates for both biomarker development and therapeutic targeting.

Conflicts of interest

There are no conflicts of interest in this work.

Acknowledgement

I appreciate the Trakya University Technology Research Development Application and Research Center (TUTAGEM) for providing all the lab equipment.

Ethical Approval Statement

Not applicable

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