



# Serum metabolomic signatures of cutaneous malignancies identified by untargeted profiling

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## Abstract

**Background and aims.** Skin cancers are among the most common malignancies worldwide, and improved non-invasive diagnostic tools are needed to support clinical decision making. Metabolomics offers a promising approach by capturing systemic biochemical alterations associated with tumor presence.

**Methods.** In this study, we performed untargeted serum metabolomic profiling based on high-performance liquid chromatography coupled with mass spectrometry in 85 individuals, including 49 with basal cell carcinoma, 19 with squamous cell carcinoma, 8 with melanoma and 9 with benign lesions.

**Results.** The analysis yielded 98 polar metabolites and 53 lipophilic metabolites that passed quality filtering. Thirteen metabolites showed significant differences between the cancer and benign groups, including lysophosphatidylcholine (20:3), N-palmitoyl tryptophan, serotonin, and fumaric acid. Principal component analysis demonstrated clear separation between malignant and benign groups, while cancer samples displayed wider metabolic variability consistent with tumor heterogeneity. Integrating principal component scores into a linear discriminant model yielded an area under the ROC curve of 0.98 corresponding to a sensitivity of 96% and a specificity of 67%. Pathway enrichment analysis revealed consistent alterations in mitochondrial electron transport, amino acid metabolism, the urea cycle, and the Warburg effect, indicating that common metabolic pathways are perturbed across cutaneous malignancies.

**Conclusions.** These findings show that serum metabolomics can distinguish cancer from benign skin lesions and highlight metabolic signatures that may serve as potential biomarkers for non-invasive assessment of skin cancer.

**Keywords:** metabolomics, skin cancer, cutaneous malignancies, basal cell carcinoma, squamous cell carcinoma, melanoma

## Background and aims

Skin cancers constitute a substantial share of the global cancer burden, with approximately one in three newly diagnosed malignancies falling into this category [1]. Their impact continues to grow, as both melanoma and keratinocyte carcinomas, encompassing basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), have shown a persistent rise in incidence over recent decades [1]. BCC is by far the most frequent subtype, representing about 75% of all cases [2]. By contrast,

invasive melanoma accounts for only around 2% of skin cancer diagnoses yet remains the primary driver of mortality, responsible for nearly 80% of deaths attributed to skin cancer [3].

The diagnosis of skin cancer relies mainly on clinical examination and dermatoscopy, approaches with variable sensitivity and specificity ranging from 67% to 98%, and with accuracy that can differ considerably across practitioners [4]. As a result, examination of excised lesions through histopathology remains the gold standard. This situation creates a

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dilemma, since many nonsurgical treatments depend on an accurate diagnosis despite the absence of histopathological confirmation, a challenge commonly seen with approaches such as photodynamic therapy, cryotherapy, and topical agents including fluorouracil [4–9]. Consequently, there is growing interest in non-invasive diagnostic tools to support effective decision making in the management of these increasingly common cancers [10].

Metabolomics has emerged as a promising strategy to address this unmet diagnostic need by capturing the small molecule biochemical landscape associated with tumor presence [11]. By providing a comprehensive snapshot of systemic metabolic alterations, metabolomics offers the potential to reveal circulating fingerprints that distinguish malignant from benign lesions, complement clinical evaluation, and reduce reliance on tissue-based confirmation. These metabolic signatures, accessible through minimally invasive blood-based analysis, open the possibility of developing objective biomarkers that enhance diagnostic accuracy. For the moment, serum metabolomic profiling has been reported in the case of melanoma [12–16], or in the case of melanoma and SCC [15]. However, there is limited experience concerning the blood metabolomic landscape across the major skin cancer types.

The present study addresses this gap by applying comprehensive metabolic analysis to serum samples from control subjects and individuals diagnosed with BCC, SCC, and melanoma. Combining the profile of polar metabolites and lipids based on liquid chromatography coupled with mass spectrometry (LC-MS), this work aims to elucidate shared metabolic signatures that support the development of clinically meaningful diagnostic biomarkers for skin cancer.

## Methods

### Patients

We enrolled patients who attended the Department of Dermatology at the County Emergency Clinical Hospital of Cluj-Napoca, Romania, between February 2023 and September 2024. Eligibility was based on a clinical suspicion of skin cancer established through clinical examination and dermatoscopy. Participants with a documented history of malignancies were excluded. Individuals with benign lesions identified during routine visits, mainly nevi, were enrolled as the control group. All clinical assessments were carried out by dermatologists with more than ten years of professional experience.

The study was approved by the Ethics Committee of the Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca (approval no. 44/31.03.2023), and of the County Emergency Clinical Hospital Cluj-Napoca (approval no. 31186/5.07.2023), with written informed consent obtained from each participant. After surgical excision, tumors were processed as formalin fixed paraffin

embedded specimens and stained with hematoxylin and eosin. A pathologist examined the tissue sections and confirmed the histopathological diagnosis. No pathology assessment was performed for nevi since these lesions were not excised.

### Sample processing

Blood samples were obtained from each patient, and serum was prepared according to the standard internal protocol. For metabolite extraction, a 0.25 mL aliquot of serum was combined with 1 mL of a methanol-acetonitrile mixture (2:1) to precipitate proteins. After vortexing for 30 seconds, the mixtures were kept at  $-20^{\circ}\text{C}$  for 24 hours. Following thawing, samples were centrifuged at 12,500 g for 10 minutes and the resulting supernatants were filtered through 0.2  $\mu\text{m}$  nylon filters. The clarified extracts were transferred to autosampler vials for metabolomic analysis.

### LC-MS

The LC-MS analysis was performed using an ultra-high-resolution quadrupole time-of-flight mass spectrometer (Daltonics MaXis Impact, Bruker GmbH) coupled to a high performance liquid chromatography system (UltiMate 3000, Thermo Scientific) equipped with a quaternary pump (Dionex UltiMate 3000 Pump, Thermo Scientific). The separation was carried out on a reversed phase  $\text{C}_{18}$  column (Acclaim UPLC C18, Thermo Scientific) using a gradient elution. The mobile phases were 0.1 % formic acid in water (solvent A), and 0.1 % formic acid in acetonitrile (solvent B). The total run was 15 minutes, the flow rate was 0.8 mL per minute, and the injected volume was 5  $\mu\text{L}$ . The column temperature was kept at  $28^{\circ}\text{C}$ .

The gradient program was 90% to 85 % A (0 - 3 minutes), 85% to 50 % A (3 - 6 minutes), 50% to 30 % A (6 - 8 minutes), 30% to 10% A (8 - 12 minutes), followed by return to 90 % A at minute 15. The internal standard was a 0.5 mg/mL doxorubicin hydrochloride solution (parent ion  $m/z= 544.1360$ ). Mass spectra were collected between 50 and 1000 Da. In parallel QC samples were analyzed, for reproducibility. All measurements were done in duplicate. The nebulizing gas pressure was 2.8 bar, the drying gas flow was 12 L/minute, and the drying temperature was  $300^{\circ}\text{C}$ . Calibration with sodium formate was performed before each run.

### Data processing and statistics

Preprocessing in Data Analysis v. 4.2 included registration of total ion chromatograms, conversion to base peak chromatograms, and extraction of compound spectra using the Find Molecular Features algorithm.

Peaks with retention times under 0.3 minutes, intensities below 3000 units, signal to noise ratios below 3, and  $m/z$  values above 600 Da were removed. Alignment of  $m/z$  features was performed using the NEAPOLIS online platform ([www.bioinformatics.org/bioinfo-af-cnr/NEAPOLIS/](http://www.bioinformatics.org/bioinfo-af-cnr/NEAPOLIS/)).

All data analysis was performed in R (version 4.4.2.). To ensure high data quality, samples were retained only if

at least 70% of metabolite intensities were non-missing. Metabolites were retained if no more than 30% of values were missing across samples. Metabolite intensities were log transformed and autoscaled.

To identify metabolites differing between cancer and benign groups, the two-tailed Student's t-test was performed for each metabolite. For each comparison, fold change was computed as the ratio of mean cancer over mean benign values. Metabolites meeting both a P value threshold of 0.05 and an absolute fold change above 1.1 were considered significant.

Principal component analysis (PCA) was used to explore global metabolic variation. Missing values were imputed by the median for each metabolite, and variables were scaled to zero mean and unit variance. PCA was performed using the `prcomp()` function from the `stats v. 4.4.2` package on the selected metabolites.

To evaluate the discriminatory power of the metabolomic profiles, linear discriminant analysis was performed on PCA scores derived from significant metabolites. Linear discriminant analysis was implemented using the `lda` function from the `MASS v. 7.3-61` package. The number of principal components included in the model was defined as the smallest number required to explain at least 80% of the cumulative variance. Posterior probabilities from the fitted model were used to assign each sample a predicted cancer or benign classification. Model performance was evaluated using both training accuracy and leave-one-out cross validation executed using the `CV = TRUE` option, producing predictions for each sample when treated as an independent test case. Confusion matrices were generated for cross-validated predictions. ROC curve analysis was conducted using the `pROC v. 1.19.0.1` package, with posterior probabilities for cancer used as predictors. The area under the ROC curve (AUC) was calculated to quantify threshold-independent classification performance.

Pathway enrichment analysis was performed using the `MetaboAnalyst 6.0` online tool (<https://www.metaboanalyst.ca/>) based on the `SMPDB` database.

## Results

The study analyzed 85 samples in total, corresponding to 9 controls, 49 BCC, 19 SCC, and 8 melanoma cases. After filtering compounds that were present in at least 70% of the patients, the metabolomic profiling of serum yielded 98 polar compounds and 53 lipophilic compounds (Figure 1A). Next, we ranked metabolites based on their ability to distinguish between control and cancer samples, identifying 13 compounds that met the selection criteria (two tailed Student's t test,  $P < 0.05$  and fold change larger than 1.1) (Figure 1B and Table I).

PCA was next performed to evaluate global metabolic variation and to assess whether the serum metabolome could separate cancer from benign samples. Using all metabolites that passed quality filtering, the PCA score plot (principal component 2 versus principal component 3) showed a clear distinction between benign samples and those derived from BCC, SCC, or melanoma (Figure 1C). Cancer samples displayed wider dispersion, consistent with metabolic heterogeneity across tumor types, while benign samples formed a more compact cluster.

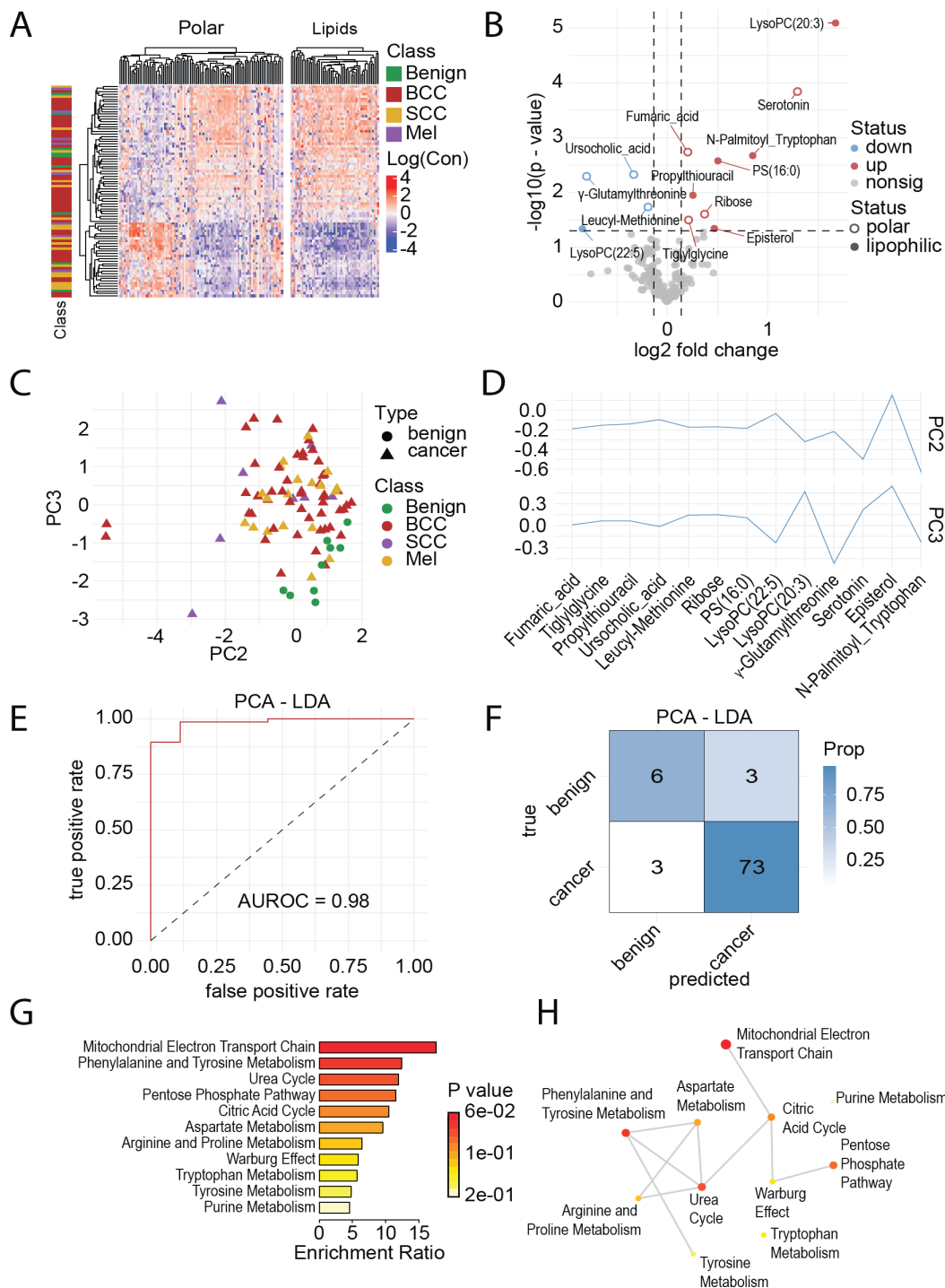
To identify the variables driving this separation, the loading vectors were examined (Figure 1D). Several metabolites that were highlighted in the univariate analysis, including lysophosphatidylcholine (20:3), N-palmitoyl tryptophan, serotonin, and fumaric acid, contributed strongly to the principal components, supporting their role as major determinants of the metabolic divergence between cancer and benign groups.

We then integrated PCA with linear discriminant analysis to evaluate the diagnostic potential of the serum metabolome. For this model, we used the first seven principal components, which together accounted for more than 80% of the total variance in the dataset. The model yielded an area under the ROC curve of 0.98 (Figure 1E). Leave-one-out cross-validation confirmed the robustness of the model, correctly assigning 73 of 76 cancer samples (96% sensitivity) and 6 of 9 benign samples (67% specificity) (Figure 1F).

Pathway enrichment analysis of the 13 discriminatory metabolites revealed alterations in central metabolic processes, including mitochondrial electron transport, amino acid metabolism, the urea cycle, and the Warburg effect (Figure 1G and 1H). These findings point to broad shifts in energy production, redox balance, and nitrogen handling in patients with skin cancer, reflecting the systemic metabolic footprint of cutaneous malignancy.

**Table I.** Classification of the 13 discriminatory metabolites according to their chemical characteristics, grouped as polar or lipophilic compounds.

Compound	Classification
Fumaric acid	Polar
Ribose	Polar
Tiglylglycine	Polar
Propylthiouracil	Polar
Serotonin	Polar
$\gamma$ -Glutamylthreonine	Polar
Leucyl-Methionine	Polar
Episterol	Lipophilic
Ursocholic acid	Lipophilic
N-Palmitoyl Tryptophan	Lipophilic
Phosphatidylserine (16:0)	Lipophilic
Lysophosphatidylcholine (20:3)	Lipophilic
Lysophosphatidylcholine (22:5)	Lipophilic



**Figure 1. Serum metabolomic profiling distinguishes cancer from benign skin lesions and identifies discriminatory metabolic signatures.** (A) Heatmap of scaled intensities for polar and lipid metabolites across benign, basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma samples. (B) Volcano plot showing  $\log_2$  fold change versus statistical significance for cancer versus benign comparisons. Thirteen metabolites met the selection criterion (two tailed Student's t test,  $P < 0.05$ ). (C) Principal component analysis (PCA) score plot (PC2 vs PC3) illustrating separation between cancer and benign samples and distribution across diagnostic classes. (D) PCA loading profiles for PC2 and PC3, indicating the metabolites contributing most strongly to group separation. (E) Receiver operating characteristic (ROC) curve for the Principal component analysis coupled to linear discriminant analysis (PCA-LDA) classification model. (F) Confusion matrix from leave-one-out cross-validation of the PCA-LDA model. (G) Pathway enrichment analysis of discriminatory metabolites, highlighting significantly enriched metabolic pathways. (H) Network representation of enriched pathways, showing functional relationships among metabolic processes altered in cancer.

## Discussion

In this study we have performed metabolic profiling of serum from patients with skin cancer across the three major histological types and identified 13 metabolites that can distinguish cancer samples from controls with a sensitivity of 96% and a specificity of 67% (AUC = 0.98). Collectively, these results show that serum metabolomics captures biochemical changes associated with cutaneous malignancies and support its potential utility in non-invasive diagnostics.

This study expands the application of metabolomics across cutaneous malignancies by incorporating the three major histological subtypes (BCC, SCC, and melanoma), whereas previous work has largely concentrated on melanoma. For example, in one of the largest studies to date, Morsy et al. analyzed 142 serum samples, including 87 melanoma patients in the exploratory cohort, 37 additional melanoma patients in the validation cohort, and 18 healthy controls [15]. They identified six robust biomarkers, notably muramic acid and several amino sugar-related metabolites, with muramic acid achieving AUC values of 0.96 in stage 1 melanoma, 0.91 in stage 2 melanoma, and 0.99 in advanced melanoma. A major strength of that study was the independent validation enabled by its relatively large dataset. Likewise, Pena Martín et al. performed untargeted profiling on 105 discovery samples from 26 healthy individuals and 79 melanoma patients, followed by validation in an independent set of 12 stage 1 melanoma patients and 6 controls [16]. Their analysis revealed three lipid metabolites, phosphatidylethanolamine (18:2), phosphatidylcholine (18:2), and hexosyl phosphatidylethanolamine (18:2), and the resulting three-metabolite model achieved an accuracy of 0.94 and a precision of 0.92 in validation. Another study by Bayci et al., involving 72 participants (26 with advanced melanoma and 46 matched controls), identified a diagnostic panel comprising phosphatidylcholine diacyl C40:3, carnitine, octanoyl-L-carnitine, ethanol, and methylmalonyl-L-carnitine [12]. Their logistic regression model yielded an AUC of 0.82, with 100% sensitivity and 56% specificity. Notably, however, this study focused solely on advanced melanoma, whereas our work encompasses cutaneous cancers across all stages. A smaller study by Fukumoto et al. evaluated serum metabolomes in both melanoma and SCC but included few patients and did not apply supervised classification [17]. Overall, our results suggest that the diagnostic performance rates reported in prior melanoma-centered investigations can be extended to BCC and SCC.

Major contributors to this separation were lysophosphatidylcholine (20:3), N-palmitoyl tryptophan, serotonin, and fumaric acid, reflecting disturbances in mitochondrial electron transport, amino acid metabolism, the urea cycle, and the Warburg effect. The observed metabolomic changes are congruent with the metabolic reprogramming typically seen in malignant transformation,

including enhanced glycolysis, altered lipid turnover, and disrupted mitochondrial function [18].

Several limitations should be considered when interpreting the results of this study. First, although the overall cohort included 85 participants, the distribution across diagnostic groups was uneven, with smaller numbers of patients with melanoma and SCC. This limited the power to detect subtype specific metabolic signatures and may have contributed to the wider dispersion of cancer samples observed in the PCA. Second, the study included only patients from a single center, which may introduce demographic or geographic bias in metabolic profiles. External validation in independent and more diverse populations will be essential to confirm the generalizability of the findings. Finally, while the LC-MS platform provided broad coverage of both polar and lipophilic metabolites, it did not allow for absolute quantification, and certain classes of metabolites such as very long chain lipids or volatile compounds may have been underrepresented. Despite these constraints, the study provides a meaningful step toward characterizing the systemic metabolic landscape of the major skin cancer types and supports further development of metabolomic biomarkers in dermatologic oncology. These results extend the observations from our recent metabolomic analysis of BCC risk groups [19].

## Conclusion

This study demonstrates that serum metabolomics captures biologically meaningful metabolic alterations shared across the major forms of skin cancer. By integrating LC-MS profiling with multivariate analysis, we identified a panel of 13 metabolites that reliably distinguished malignant from benign lesions and revealed coordinated changes in lipid remodeling, amino acid metabolism, mitochondrial function and nitrogen handling. The supervised classifier achieved an AUC of 0.98 corresponding to a sensitivity of 96% and a specificity of 67%, underscoring the potential of circulating metabolic markers as supportive tools in dermatologic decision making. Although external validation and larger subtype specific cohorts are needed, the present work expands the metabolomic landscape beyond melanoma alone and provides evidence that common metabolic pathways are perturbed across diverse cutaneous malignancies. These findings support continued exploration of serum metabolomics as a non-invasive approach to improve early detection, guide clinical evaluation and enhance understanding of the systemic biology of skin cancer.

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