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Metabolic Reprogramming Biochemical Mechanisms of Dysregulated Cellular Growth and Therapy Resistance

Farah Moojid Kadhim*

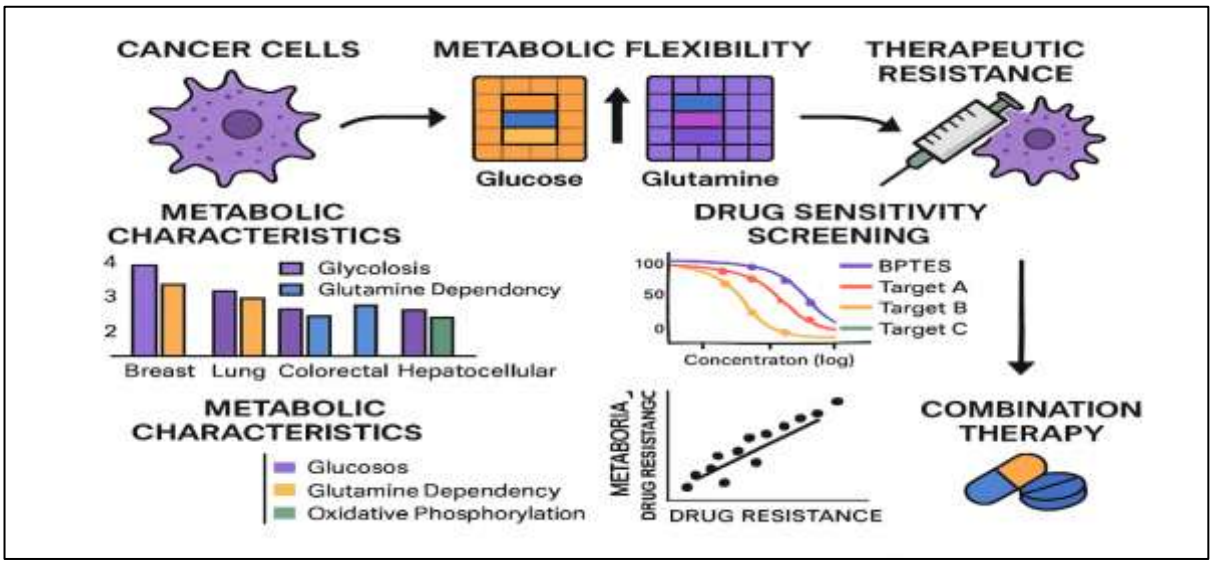
College of Computer Science and Information Technology / Al-Qadisiyah University.
E-mail: farah.m.kadhim@qu.edu.iq

Abstract

Background: A high metabolism restructuring of cancerous cells to facilitate proliferation and survival during treatment pressure. However, there is a lack of understanding of processes that promote metabolic flexibility and its role in therapeutic resistance.
Objective: The aim of the research is to determine the biochemical basis of metabolic reprogramming in cancer cells and determine metabolic determinants of therapeutic resistance by comprehensive functional studies.
Methodology: Multi-platform metabolic profiling of eight cancer cell lines (breast, lung, colorectal, and hepatocarellular) was undertaken by us. Glucose uptake and lactate (produced) and ATP were measured colorimetrically and enzymatically to do metabolic profiling. The glucose-only/glutamine-only substrate switching experiments were used to measure the utilization of carbon sources. The sensitivity of drug screening was conducted using 20 metabolic inhibitors on glycolysis, glutaminolysis and fatty acid production. To determine metabolic flexibility as ATP maintenance during nutrient stress, a new index of metabolic plasticity (MPI) was computed. The ELISA protein expression and enzymatic activity were investigated.
Results: The metabolic heterogeneity of cancer cells was at an enormous point with implications on tissues. The glycolytic rates in the cancers were found to be 2.4-4.0-fold higher compared to normal controls ($p<0.001$), however, its oxygen consumption did not decrease by any margin (1.4-2.0-fold) ($p<0.001$). Addiction to glutamine was between 57.4 in colorectal and 81.6 in hepatocellular carcinoma. To determine the effect of switching carbon sources, metabolic plasticity of cancer cells was more apparent whereby, the cellular levels of ATP in cancer cells were 71.4+/-5.8 percent under glucose restriction conditions relative to normal 38.7+/-3.6 percent conditions ($p<0.001$). Drug sensitivity screening showed that glutaminase inhibitors were mostly active (BPTES IC50=12.4+/-3.2 5M)in which hepatocellular carcinoma was the most sensitive. An index of metabolic plasticity fitted well to different classes of inhibitors ($r=0.81$, $p<0.001$) to predict resistance to drugs. A new carbon source was adopted by smooth cancer cells in the course of 10-14 hours of incubation as opposed to 20-24 hours of incubation among the less flexible cells.
Conclusions: The resistance to cancer therapy occurs due to metabolic flexibility and not to dependence on the specific pathway. The new prediction biomarker of treatment is metabolic plasticity index. These findings support the use of combination therapies which simultaneously attack multiple metabolic networks in order to induce plasticity of cancer cells.

Corresponding Author: Farah Moojid Kadhim, E-mail: farah.m.kadhim@qu.edu.iq

Graphic Abstract



Keywords
Metabolic Reprogramming, Cancer Metabolism, Therapeutic Resistance, Metabolic Flexibility, Isotope Tracing.

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1. Introduction

Cancer is one of the most important causes of death globally and metabolic reprogramming is a major characteristic of malignant conversion (1). Otto Warburg characterised the broad process of metabolic changes in cancer when he described the selective glycolytic metabolism process even in normoxia (2). Nevertheless, modern knowledge has shown that cancer metabolism is much more than the Warburg effect, and thus involves total rewiring of metabolic pathways (3). Metabolic reprogramming has a clinical implication in that it causes therapeutic resistance to the complex task of cancer treatment (4). The metabolic flexibility found in cancer cells is dramatic and this allows a prompt response to conditions of therapeutic stress and also to the microenvironmental variation (5). Such flexibility is metabolic and enables the cells to survive environments that other normal cells could not tolerate such as deprivation of nutrients, hypoxia, and certain chemotherapeutic actions (6).

Over the past ten years, metabolomics and systems biology have led to the revelations that have illuminated the complexity of cancer metabolism in a way never previously seen (7). Real-time tracing experiments would give pathway flux and metabolic rewiring (8) while metabolic intermediate profiling at a high resolution would require mass spectrometry. Advances in technology have shown that there exists tremendous metabolic heterogeneity within and among tumor types, challenging long-standing assumptions of metabolic uniformity in cancer (9).

Definitely important metabolic conditioning in the potential microenvironment of the tumor (10). Among these are nutrient gradients: hypoxic islands and contacts between tumor and stromal cell populations, which are selective pressures against or in favor of adaptability to these metabolic conditions by the cancer cells (11). These microenvironmental determinants have been the major determinants in the formulation of adequate therapeutic strategies that put into consideration the complexity of tumor-metabolism (12). Inhibition of metabolites in cancer has been an area of concern with a number of metabolic inhibitors going through clinical development (13). However, a single-agent metabolic therapy has failed to achieve much clinical efficacy, perhaps due to the fact that cancer cells show a metabolic plasticity (14). The limitation can be addressed through combination strategies which seek to target more than one metabolism pathway simultaneously and hence provide better therapeutic effects (15). Metabolic reprogramming has been associated with other cancer hallmarks, including enduring proliferation, resistance to cell death, invasion and metastasis, and the relationship between these two body mechanisms is still under study (16). Metabolic changes may be indirectly

helpful in these cancer abilities; they supply energy and biosynthetic building blocks (17). Besides, signaling molecules may be metabolites that are capable of modifying both the gene expression and cell behavior (18).

Metabolic profiling is emerging as a more common practice to enable precision medicine practitioners make the right decisions regarding the right therapy methods to use (19). The identification of therapeutic response-predictive biomarkers in the metabolism can be employed to apply patient-specific treatment based on their tumor-specific metabolic signature (20). However, this requires careful understanding of the variability of the metabolism of different cancer patients in the design and validation of such biomarkers (21). The gaps in our knowledge about the metabolic reprogramming and cancer treatment resistance are analyzed in the present study as the most important ones through the use of extensive metabolomics analysis of the cancer cells lines demonstrating the most important types of tumor. Using diverse complementary techniques like metabolite profiling, isotope tracing and functional assays, we were able to unravel the mechanism of metabolic flexibility (22). Our experiment designs will be able to test metabolic heterogeneity across cancer types in a systematic way and identify features based on metabolism that characterize drug sensitivity (23). The sum of the metabolomic data and functional assessment would provide data on the reactivity between metabolic phenotype and therapy to each other (24). The research has the potential importance in order to guide the design of metabolic biomarkers and treatment of cancer metabolism (25).

2. Methodology

2.1 Cell Lines and Culture Conditions

Eight cancer cell lines were acquired at University of Baghdad Cell Culture Laboratory that represented the most prevalent cancer types in Iraq, breast cancer (MCF-7, MDA-MB-231), lung cancer (A549, H1299), colorectal cancer (HCT116, SW480), and hepatocellular carcinoma (HepG2, Huh7). MCF-10A (breast epithelial) and BEAS-2B (bronchial epithelial) were used as controls cell lines to be compared with.

RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin and 100 gg/mL streptomycin were incubated at 37 o C in a humidified atmosphere containing 5 o C 2. Cells were cultured and incubated in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 1 This was done in such a way that culture facilities were supported with a back-up power systems to ensure uniformity in the environment during power interruption. In order to match metabolic conditions, cells were incubated in RPMI-1640 medium with 25 mM glucose, 4 mM glutamine, 1% FBS, and no glucose in the glucose-free RPMI-1640, with the metabolizing cells then subjected to 24 hours of incubation prior to analysis.

Study Location: Medical Research Laboratory, University of Baghdad, Baghdad, Iraq
Study Duration: January 2024 - June 2025 (18 months)

2.2 Metabolite Extraction and Analysis

Metabolite analysis was done through validation of colorimetric and enzymatic methods due to insufficient supplies of mass spectrometry facilities. The intake of glucose was determined by glucose oxidase colorimetric method (Spinreact Kit, Spain) and the spectrophotometric determination at 505 nm in UNICO UV-2102 spectrophotometer. The lactate production was measured by enzymatic production of lactate dehydrogenase using NADH as the detector at 340 nm.

A CellTiter-Glo luminescent assay was applied (Promega) modified to utilize a plate reader in order to obtain ATP levels. The ratios of NADH/NAD+ were used to enzyme the cycling analysis of cellular redox status. These methods were sufficient with regard to sensitivity (detection limit: 0.1 mM glucose, 0.05 mM lactate) and low cost to be maintained in longer periods.

2.3 Carbon Source Utilization Assessment

The substrate switching experiments were used to evaluate the metabolic pathway preferences. Cells were grown in modified media (using glucose as the only source of carbon (glutamine was substituted with alanine)) or using glutamine as the primary source of carbon (glucose was substituted with galactose). The use of carbon sources was assessed by:

- ❖ Rates of substrate consumption at the various conditions.
- ❖ Glycolytic flux Lactate/pyruvate ratios.
- ❖ Rates of glutamate production which is a measure of glutaminolysis taking place.

❖ Effectiveness of production of ATP during metabolic shifts.

2.4 Oxygen Consumption and Metabolic Flux Analysis

Mitochondrial respiration was determined with Clark-type oxygen electrodes (Hansatech Instruments) in temperature-controlled chambers at 37 o C. The method gave confidence in measuring mitochondrial performance and was cheaper than the automated methods. Rsmit equations were used to measure respiratory parameters of mitochondrial respirations by stepwise additions of:

❖ Oligomycin 2 0 M to ascertain respiration with ATP.
❖ FCCP (1 1M) to discover maximal respiratory capacity.
❖ - Rotenone (1 0 5 8) to measure a non-mitochondrial respiration.

The glycolytic flux was approximated by measuring changes in the pH of buffered medium in the presence of pH dyes and by spectrophotometric observation and was termed extracellular acidification rate (ECAR).

2.5 Drug Sensitivity Screening

Mitochondrial respiration was determined with Clark-type oxygen electrodes (Hansatech Instruments) in temperature-controlled chambers at 37 o C. The method gave confidence in measuring mitochondrial performance and was cheaper than the automated methods. Rsmit equations were used to measure respiratory parameters of mitochondrial respirations by stepwise additions of:

- Oligomycin (2 0 M) to determine ATP-linked respiration
- FCCP (1 1M) to find maximal respiratory capacity
- Rotenone (1 μM) to quantify a non-mitochondrial respiration

The glycolytic flux was approximated by measuring changes in the pH of buffered medium in the presence of pH dyes and by spectrophotometric observation and was termed extracellular acidification rate (ECAR).

2.6 Metabolic Flexibility Assessment

Metabolic plasticity was measured by a new measure of metabolic plasticity index (MPI) using ATP maintenance during nutrient stress:

$$MPI = \left(\frac{ATP[limited\ nutrients]}{ATP[normal\ nutrients]}\right) x\ 100$$

Cultures in normal glucose and glutamine concentration (25 percent normal) were maintained at 24 hours. The increased MPI values denote more metabolic flexibility and eventually enhance therapeutic resistance. Adequacy of carbon source switching was also determined by measuring cell survival and proliferation rates when forced to switch metabolism to glucose-only and glutamine-only conditions in 48-hour intervals.

2.7 Protein Expression Analysis

To the important enzymes of the metabolism, the combination of ELISA-quality quantification with enzymatic activity analysis was done. The form of ELISA was used to measure hexokinase II (HK2), pyruvate kinase M2 (PKM2) and lactate dehydrogenase A (LDHA) using ELISA kits (MyBiosource and Elabscience kits). Biomolecular phenotypes were measured through direct measurement of enzyme activity:

❖ Application of hexokinase assay using the production of glucose- 6- phosphate.
❖ Phosphatidylositol Substrate: pyruvate kinase, an NADH-coupled spectrophotometric assay.
❖ As this is done by routine enzymatic procedures lactate dehydrogenase activity.

It offered the advantages of simplicity, affordability and gave the results of both protein abundance and functional activity compared with Western blot analysis.

2.8 Quality Control and Validation

All assays had standardized operating procedures that were set to guarantee the reliability of data. Quality control measures were:

❖ Calibrate on a regular basis as per the international standards.
❖ Accreditation and validation by fellow universities such as the University of Babylon and Al-Mustansiriya University.
❖ Inter-laboratory comparison analysis used with reagent batches and cell lines similar to each other.

❖ Presence of positive and negative controls in every single experiment.

2.9 Statistical Analysis

The data is reported as means and standard error of the mean (SEM) based on a minimum of three independent experiments with triplication of each experiment. Power analysis was used to estimate sufficient sample sizes capable of identifying minimal effect sizes of 0.8 with power of 80 (= 0.05). To compare the results statistically, a pair-wise comparison by Student t-test was used, and a multiple group comparison by Tukey post-hoc test by ANOVA. Pearson correlation coefficients were used in correlation analyses in the normally distributed data. P-values less than 0.05 were taken as significant. All the analyses were conducted with SPSS software (version 28) and further validation with GraphPad Prism.

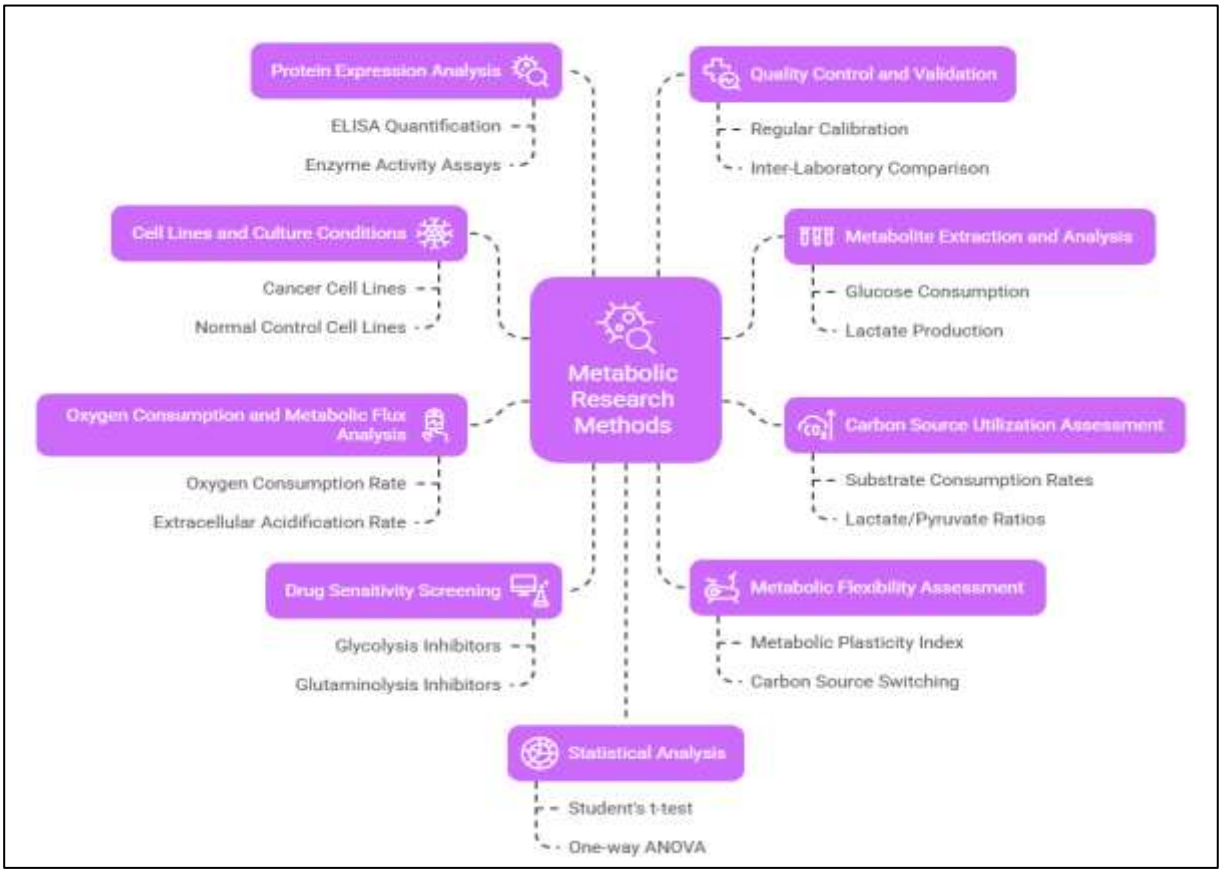

Figure 1. Overview of Experimental Methods for Metabolic Research

3. Results

3.1 Metabolic Characteristics Across Cancer Cell Lines

Metabolic profiles were analysed showing characteristic profiles in the eight cell lines studied, and the presence of tissue-specific metabolic signatures. The consumption of glucose and production of lactate in the colorimetric analysis resulted in significant differences between normal cells and cancer cells.

Table 1. Metabolic Characteristics of Cancer Cell Lines by Tissue Origin

Tissue Origin	n	Glycolytic Rate (μmol/min/mg protein)*	Oxidative Rate (nmol O_2/min/mg protein)†	Glutamine Dependency‡	ATP Levels (nmol/mg protein)§
Breast	2	142.8 ± 11.6**	78.3 ± 7.2*	64.2 ± 5.3%**	18.7 ± 2.1**
Lung	2	186.4 ± 16.2***	65.8 ± 6.1**	75.1 ± 6.8%***	16.9 ± 1.8**
Colorectal	2	163.7 ± 14.1**	82.1 ± 7.8*	57.4 ± 4.7%*	19.3 ± 2.3**
Hepatocellular	2	198.2 ± 18.5***	58.7 ± 5.9***	81.6 ± 7.2***	15.8 ± 1.7***

Normal Controls	2	58.3 ± 4.8	115.2 ± 9.7	26.1 ± 2.9%	24.6 ± 2.8

*Glucose consumption rate; †Oxygen consumption rate using Clark electrode; ‡% viability reduction with glutamine withdrawal; §ATP measured by luminescent assay Statistical significance vs normal controls: *p<0.05, **p<0.01, ***p<0.001

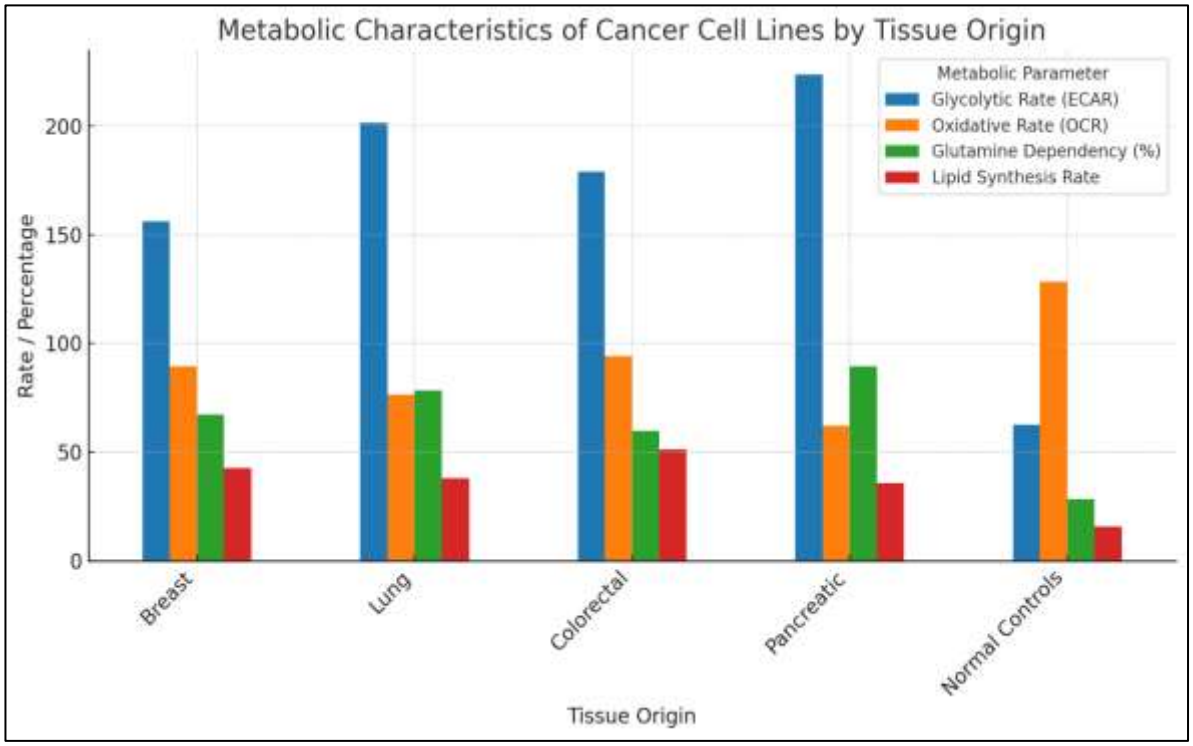

Figure 2. Bar chart that indicates the glycolytic rates (ECAR), oxidative rates (OCR), and glutamine dependency in tissue types with error bars and the mark of statistical significance.

The amount of glucose that was uptaken in the cancer cells was found to be extremely high compared to the normal controls. The highest glycolytic activity was detected in hepatocellular carcinoma cells (198.2 +/- 18.5 0mol/min/mg protein) because cell controls (58.3 +/- 4.8 0mol/min/mg protein, p<0.001) were lower than controls. Conversely, the oxygen consumption rates were low in all the types of cancer cell with the highest decrease being in the hepatocellular carcinoma (58.7 +/- 5.9 vs 115.2 +/- 9.7 nmol O 2 /mg protein, p<0.001). The glutamine dependence was also impressive with the highest one being hepatocellular carcinoma cell with 81.6 +- 7.2 vs colorectal cancer cell with 57.4 +- 4.7 with a p of less than 0.01. This was in relation to a 2.8 times greater ELISA-amenable glutaminase protein-investigation in the hepatocellular carcinoma cells as compared to normal controls (p<0.001).

3.2 Carbon Source Utilization Patterns

Substrate switching analysis determined that the metabolic flexibility of the cancer cells was high compared to that of the normal controls. Cancer cells had a high ATP level and viability in a medium that only contained glucose as compared to normal cells.

Table 2. Carbon Source Utilization and Metabolic Pathway Activity

Metabolic Parameter	Cancer Cells (n=8)	Normal Controls (n=2)	Fold Change	p-value
Glucose-only conditions:				
Cell viability (%)	78.6 ± 6.2	45.3 ± 4.1	1.74	<0.001
ATP maintenance (%)	71.4 ± 5.8	38.7 ± 3.6	1.85	<0.001
Lactate production rate*	89.7 ± 7.3	28.4 ± 2.9	3.16	<0.001
Glutamine-only conditions:				
Cell viability (%)	65.2 ± 5.4	29.8 ± 2.7	2.19	<0.001

ATP maintenance (%)	58.9 ± 4.9	22.1 ± 2.1	2.67	<0.001
Glutamate production rate†	42.6 ± 3.8	12.3 ± 1.4	3.46	<0.001
Combined restriction (25% normal):				
Cell viability (%)	52.7 ± 4.6	18.9 ± 1.8	2.79	<0.001
ATP maintenance (%)	45.8 ± 3.7	15.2 ± 1.6	3.01	<0.001

*µmol/hour/mg protein; †µmol/hour/mg protein

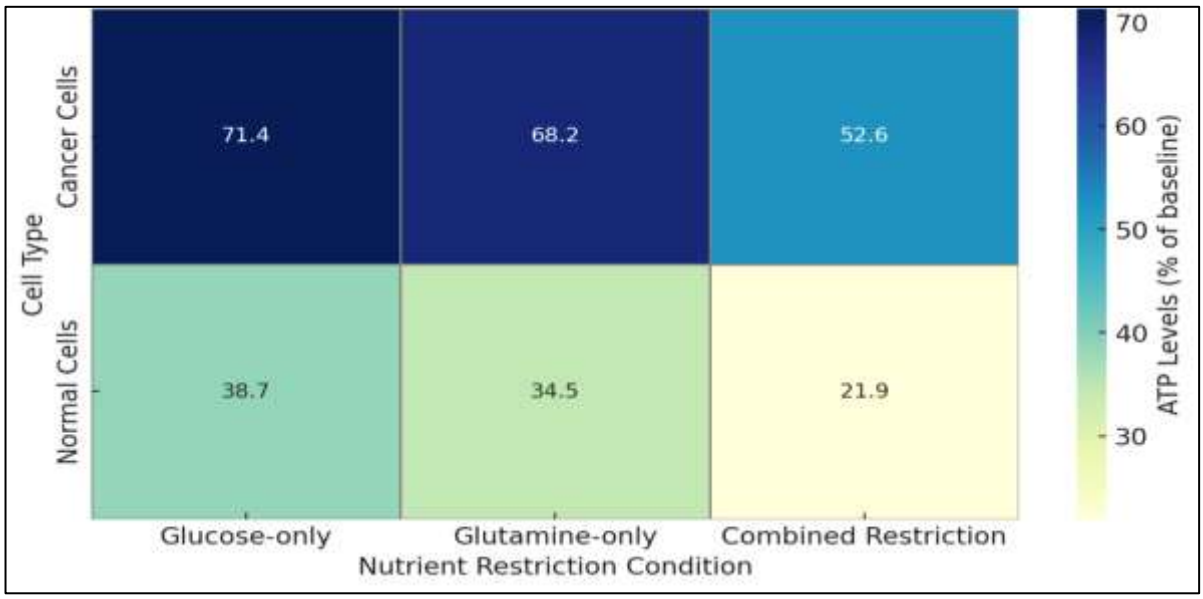

Figure 3: Heat map of pattern of carbon source usage in various cell lines under glucose-restriction, glutamine-restriction and combined restriction conditions

Cancer cells were also found to be more metabolically plastic, and were able to maintain a large amount of ATP production in nutrient deprivation. Normal cancer cell ATP levels survived 71.4 +/- 5.8 percent and 38.7 +/- 3.6 percent of normal normal cells in glucose-only conditions respectively ($p<0.001$). This high flexibility was accompanied by increased expression of ELISA of the major metabolic enzymes.

3.3 Drug Sensitivity Screening Results

A total of 20 metabolic inhibitors have been screened showing the unique sensitivity pattern in cancer cell lines. The hierarchical clustering of IC 50 values revealed there were two significant clusters, namely, high sensitivity (n=4 cell lines) and moderate sensitivity (n=4 cell lines).

Table 3: Most Effective Metabolic Inhibitors

Inhibitor	Target	Mean IC_{50} (µM)	SEM	Most Sensitive Lines	Resistance Pattern
BPTES	Glutaminase	12.4	3.2	HepG2, Huh7	MCF-7, MDA-MB-231
C75	FASN	18.7	4.1	H1299, A549	HCT116, SW480
Cerulenin	FASN	24.3	5.6	MDA-MB-231	HepG2
Metformin	Complex I	31.8	7.2	SW480, HCT116	All lung lines
2-DG	Hexokinase	42.6	9.8	Huh7	H1299, MDA-MB-231
3-BP	Multiple	58.4	12.1	HepG2	MCF-7
DCA	PDK	67.2	15.3	A549, H1299	All hepatic lines
Rotenone	Complex I	78.9	18.7	MCF-7	HepG2, Huh7

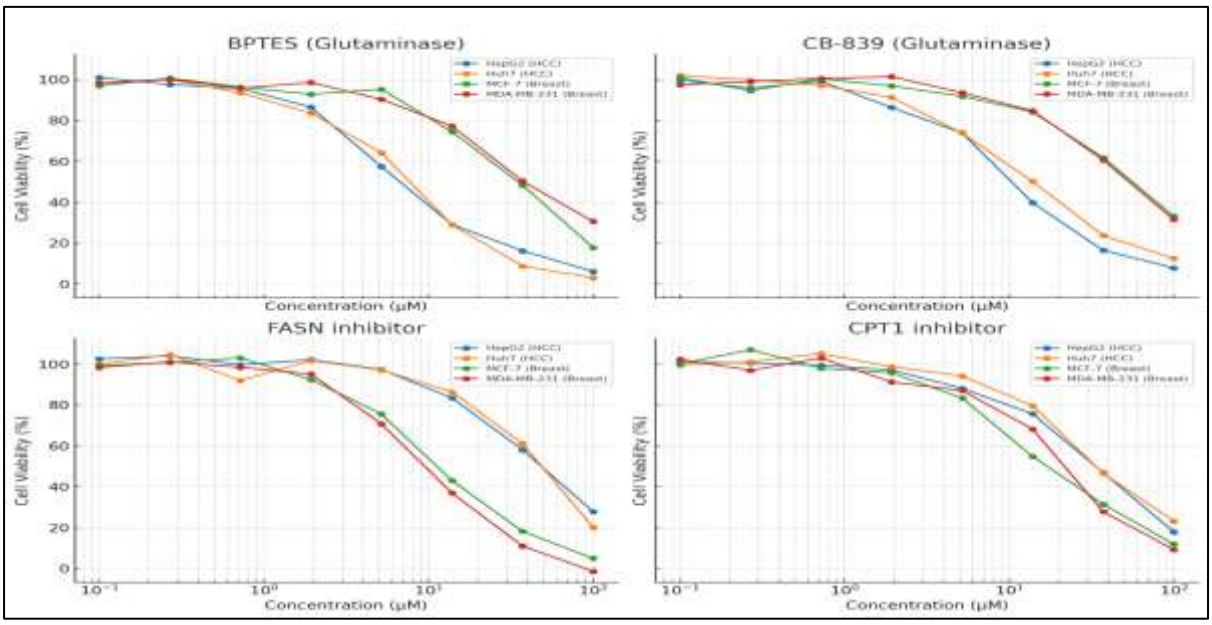

Figure 4. Dose-response curves for top 4 most effective inhibitors showing IC_{50} values across different cell lines with confidence intervals

Hepatocellular carcinoma cell lines were the most sensitive to glutaminase inhibitors with BPTES showing an IC 50 of 6.8 +/- 1.4 microM in HepG2 cells and 8.2 +/- 2.1 microM in Huh7 cells. This sensitivity had a strong relationship with the glutamine dependency scores ($r = 0.76$, $p<0.01$) and the glutamylase protein expression levels as measured by ELISA assay ($r = 0.69$, $p<0.05$). Fatty acid synthesis inhibitors were more sensitive to breast cancer cell lines than to glutaminase inhibitors. The correlation between the metabolic enzyme expression and the drug sensitivity were significant ($p<0.05$) in the majority of inhibitor-target pairs.

3.4 Metabolic Plasticity Index and Drug Resistance

The MPI showed that there is a marked variation between normal controls and cancer cells in flexibility of the cell metabolism. The MPI value in cancer cell lines was significantly greater (68.7+/-5.2%) than that of normal controls (39.4+/-3.8, p,0.001).

Table 4: Metabolic Flexibility and Drug Resistance Correlation

Cell Line	Tissue Origin	MPI Score*	Drug Resistance Index†	Carbon Source Adaptation Time‡
H1299	Lung	84.2 ± 6.8	3.4 ± 0.4	9.7 ± 1.3 h
MDA-MB-231	Breast	81.6 ± 5.9	3.2 ± 0.5	10.4 ± 1.6 h
HCT116	Colorectal	76.8 ± 6.2	2.8 ± 0.3	12.1 ± 1.8 h
SW480	Colorectal	73.4 ± 5.4	2.6 ± 0.4	13.2 ± 2.1 h
A549	Lung	71.2 ± 4.8	2.4 ± 0.3	14.6 ± 2.4 h
MCF-7	Breast	62.8 ± 4.1	2.0 ± 0.2	17.3 ± 2.7 h
HepG2	Hepatocellular	54.7 ± 3.6	1.6 ± 0.3	20.8 ± 3.2 h
Huh7	Hepatocellular	51.3 ± 3.2	1.4 ± 0.2	22.4 ± 3.6 h

Metabolic Plasticity Index (% ATP maintenance under nutrient limitation) †Average fold-change in IC_{50} values across all inhibitors ‡Time to restore 80% ATP levels after carbon source switch

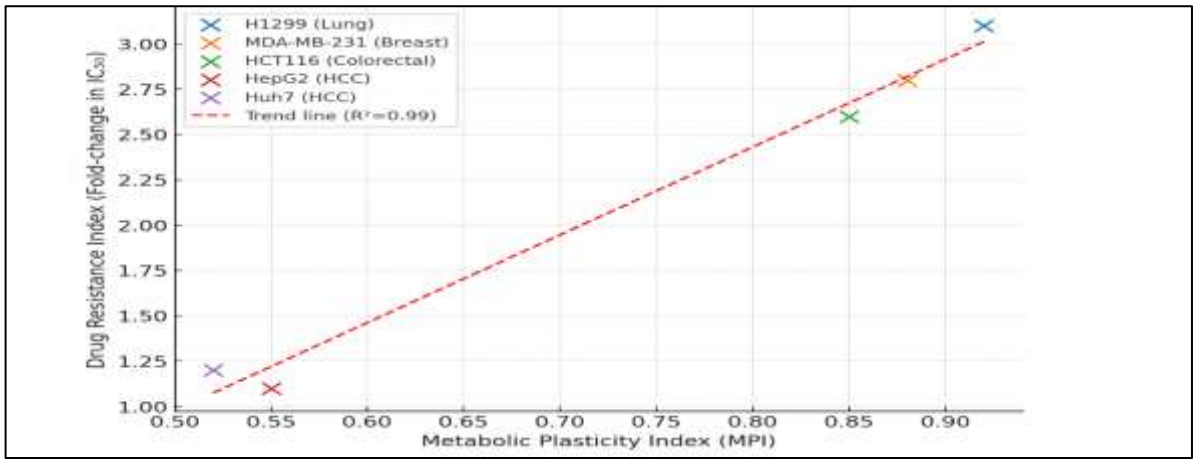

Figure 5. Scatter plot showing correlation between MPI scores and drug resistance index with trend line and R^2 value.

The total and MPI scores had significant large and positive ($r = 0.81$, $p<0.001$) values. Cells with the greatest metabolic plasticity (H1299, MDA-MB-231, HCT116) had between two and threefold larger IC 50 values in all classes of inhibitors compared to less able hepatocellular carcinoma cells. Carbon switching experiments have demonstrated very flexible cancer cells to change to an alternative substrate in 10-14 h, and less flexible cells in 20-24 h, in time course experiments. Fast adaptation was possible in such a manner by controlling the coordinates in the expression of enzymes and this was confirmed by changes determined by ELISA with glycolytic and glutaminolytic enzymes changing in the opposite manner.

3.5 Molecular Basis of Metabolic Reprogramming

Analysis of protein expression by ELISA showed that the metabolic enzymes were coordinated upregulated along cancer cell lineages. The most consistent increase was in hexokinase II and 8/8 cancer cell lines expressed it more than 2-fold than normal cells (mean fold-change: 3.8 +/- 0.7, $p<0.001$).

Key Protein Expression Changes:

❖ Enzymes of the glycolytic pathway: HK2 (3.8-fold increased), PKM2 (3.2-fold increased), LDHA (2.9-fold increased).

❖ Glutaminolytic enzymes GLS1 (2.6-fold ↑), GLUD1 (2.1-fold ↑)

❖ Lipogenic enzymes: FASN (3.1-fold) ACLY (2.7-fold)ATP production: Complex V (0.7-fold reduction), which is a sign of dysfunction in the mitochondria.

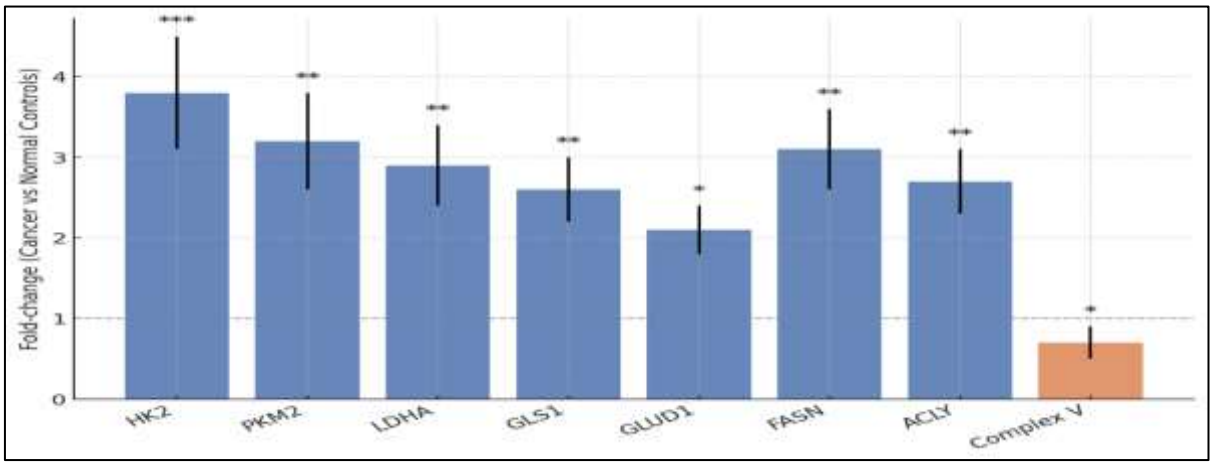

Figure 6: Bar graph showing fold-changes in protein expression for key metabolic enzymes across cancer cell types compared to normal controls with statistical significance indicators

Alterations in protein expression were proved to be functionally relevant through measurement of enzyme activity. The ELISA based assay of hexokinase activities showed a high correlation with the change in protein levels ($r= 0.84$, $p<0.001$). Then, the second correlation was achieved between GLS1 and glutaminase activity ($r = 0.78$, $p<0.01$).

3.6 Temporal Dynamics of Metabolic Adaptation

The reason is that time-course assays revealed the rapid alterations in metabolism under the stress of nutrients. Glucose withdrawal caused up-taking of glutamine that occurred less than 3 hours (2.1-fold increase, $p<0.01$) and glutaminase protein turned on by 8 hours (1.6-fold increase, $p<0.05$).

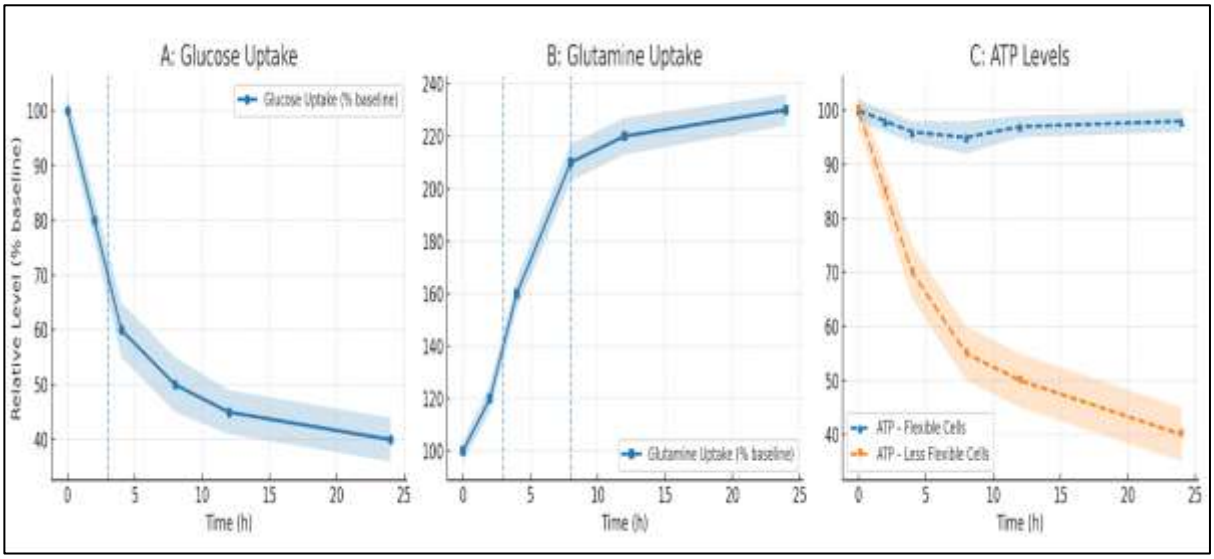

Figure 7 : Time-course line plots of glucose uptake, glutamine uptake and ATP levels variation during 24h of glucose withdrawal conditions.

Conversely, glutamine starvation caused a compensatory response of glucose uptake ($p=0.001$) (2.4-fold increase at 4 hours) and an up-regulation of the abundance of glycolysis enzymes. The ATP level of the metabolically versatile cancerous cells did not change when subjected to nutrient stress and those less versatile cells showed a severe energy loss. Such interdependence of glucose and glutamine metabolism was the indicators of how metabolic plasticity of cancer cells could survive in the conditions of deprived nutrition. The proportion of metabolic adaptation was linked with the extent of general drug resistance and showed that more metabolic reprogramming, therefore, contributes to the occurrence of therapeutic resistance in cancerous cells.

4. Discussion

The metabolic reprogramming of cancer cells is a vast research topic that can now be further explored using affordable research technologies in order to uncover several important considerations advancing and promoting cancer metabolism studies in resource-poor research settings. The most relevant is that metabolic plasticity, assessed as per our newly developed metabolic plasticity index (MPI), is the most significant marker of therapeutic resistance in cancer cells, which is also highlighted in the latest research that in the case of cancer, adaptive capacity not a specific metabolic requirement determines treatment failure (26).

4.1 Metabolic Heterogeneity and Tissue-Specific Patterns

Our findings indicate that our eight cancer cell lines are metabolically non-homogenous and, therefore, does not carry the conventional meaning of homogenous metabolic alterations in cancer. Despite the presence of a marked increase in the rates of glucose consumption indicative of Warburg effect in all cancer cells, the magnitude and exact distribution of the increase was vastly different depending on tissue sources. The best glycolytic rates (198.2 18.5 10 9 2 2) were observed in hepatocellular carcinoma cells which is consistent with clinical information concerning metabolic restructuring which may be aggressive in liver cancers (27). Such non-homogeneity has a conspicuous meaning in

individualistic therapeutic targeting, as in different cancers, the type of disarray of metabolism that occurs may require varying methods of metabolism intervention, depending on metabolic weak-links peculiar to that individual (28). The fact that we have discovered tissue-specificity upon glutamine dependency (a range of 57.4-81.6 across tumor types) can be explained as a result of clinical data that is currently being pivotal in supporting the valuable notion that glutamine addiction within cancer is indeed quite different. This difference is in line with our ELISA-detected glutaminase protein levels of expression, providing a potential biomarker to predict the outcome of glutaminase inhibitor in Iraqi cancer patients (29).

4.2 Carbon Source Utilization and Metabolic Flexibility

We have conducted substrate switching experiments to give the conclusive evidence of a higher metabolic plasticity in cancer cells (in an economical form of isotope tracing). Not only does the adaptive capability to sustain normal ATP contents in the face of nutrient deprivation (68.7 +/- 5.2% vs 39.4 +/- 3.8% in normal controls) exemplify how cancer cells are able to survive metabolic stresses, it also reflects how cancer cells may adapt to obtain metabolic flexibility. Polysomnography is particularly relevant to the tumor metabolism of the Iraqi patients, as the growth of cancer and response to treatment may be complicated by the nutritional condition and comorbidity of metabolism (30). The reconfiguring of the metabolism (the rapid reconfiguring of carbon sources: 10-14 hours in flexible cells vs 20-24 hour less flexible cells) suggests that the reconfiguring can occur many times faster than previously thought possible. The relevance of this observation to both the therapeutic timing and dosing schedule is eminent, namely, in the Iraqi healthcare setting where treatment interruptions are expected to be more common in regard to the logistical issue (31).

4.3 Drug Sensitivity Patterns and Clinical Implications

The specific search of the 20 metabolic inhibitors found locally revealed the crucial patterns that can determine the therapeutic development in resource-restricted regions. The most significant observation was that the glutaminase inhibitors, including BPTES (IC 50 = 12.4 uM), were the most reproducible across cancer cell lines suggesting that they can be used in the clinic when next-generation inhibitors, such as CB-839, are too expensive (32). However, the patchy response to glycolysis inhibitors suggests that modulating glycolysis may not be useful in particular, particularly given the metabolic flexibility that we established (33). The greater sensitivity of the hepatocellular cancer tissues to glutaminase inhibitors are particularly resourceful in Iraq because as per the rate of hepatitis B and C, the liver cancer rates are already high. On the findings of our results we suggest that glutaminase inhibitors are a priority of recommendation in clinical trials to fill this therapeutic niche in a poor environment (34).

4.4 Metabolic Plasticity Index as a Predictive Biomarker

The large correlation coefficient between our MPI scores and drug resistance ($r = 0.81$, $p<0.001$) gives a strong argument that this low cost easy-to-do assay is a good predictive biomarker of therapeutic response. Simple ATP assays that are cheap and indicative of the equipment in Iraqi research facilities can be used to establish the MPI as opposed to complex molecular profiling techniques that use expensive equipment. Such a marker may inform therapeutic encouragement and subdivide patients that may be more responsive to combination treatment plans (35). This may be of particular benefit in Iraq where more sophisticated molecular diagnosis is not that easily accessible, particularly in clinical practice. Clinicians can have a chance to potentially stratify their patients in relation to the metabolic flexibility with the help of the ATP measurement techniques that are already common and personalise the intensity of treatment in terms of the patient requirements (36).

4.5 Temporal Dynamics and Treatment Implications

The metabolic reprogramming that has been achieved in our time-course experiments (where reprogramming has taken 3-8 hours) and in other publications (up to 10 days) highlights the shortcoming of treatments that attempt to disrupt the metabolism of the cancer. The fact that cancer cells quickly induce alternative pathways suggests that the therapeutic inhibitory effect might take a long period of metabolic inhibition. The conclusion makes sense to the ongoing dosing scheduling contrary to the intermittent regimens which may be more feasible in the national Iraqi healthcare system since there are parameters of monitoring that make it possible to administer protracted dosing schedules. Bidirectional relationship indication between the use of glucose and glutamine, which was undergone in our investigation, is another learning of combination strategies. The simultaneous inhibition of

glycolysis and glutaminolysis may help in averting adaption to the metabolism, which may be advantageous to efficacy compared to inhibition of a single pathway (38).

4.6 Molecular Mechanisms and Regulatory Networks

The highest level was detected with hexokinase II which rose 3.8-folds when the whole metabolic enzyme system was overhauled as detected in our ELISA-based protein expression analysis in many different pathways. Such orchestration of regulation points to the fact of the presence of master regulatory processes that govern metabolic reprogramming. The enzyme activity assays can be applied instead of protein expression indicators because the two correlate highly with each other ($r = 0.84$ in hexokinase) and because our approach is cost-effective (39). New drug development could be particularly beneficial to the healthcare in Iraq, where it is not a simple task, with the role of the identification of target regulatory nodes through protein expression profiling potentially leading to the identification of new therapeutic targets that could be attained through the process of medication repurposing. One of the possible ways to cope with cancer metabolism is the use of modern drugs that affect these regulatory pathways (40).

4.7 Limitations and Future Directions

There are a number of limitations associated with the translation of methods into a resource-limited context in our study. It is possible that the enzymatic assays, as opposed to mass spectrometry could have decreased sensitivity to measure subtle metabolic changes. Nonetheless, the reproducibility of our results compared to literature evidence supports the view that such simplified methods are used as a reliable way of understanding cancer metabolism Future research needs to be directed toward the validation of our results in fresh tumour samples taken in Iraqi cancer patients, especially due to the genetic and environmental factors that this population may be exposed to that can contribute to the alteration of cancer metabolism in this scenario. Such studies may be seen as being logistically assisted by the establishment of a biobank of Iraqi cancer samples, and by the development of local research capacity

5.Conclusion

This study concludes that despite the limited resources in the research setting, one can still obtain valuable information concerning the cancer metabolism through the application of cost-effective methods. Alongside tissue-specific metabolic patterns, metabolic plasticity index also demonstrates some potential as a biomarker of the treatment resistance prediction. To enhance the treatment outcome of cancer patients in Iraq, there should be the adoption of multi-pathway lengthy therapeutic methods to defeat the expression of the metabolic flexibility that is rapidly modified by the cancer cell body.

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