Glucose metabolism transcriptome clustering identifies subsets of resectable lung adenocarcinoma with different prognoses

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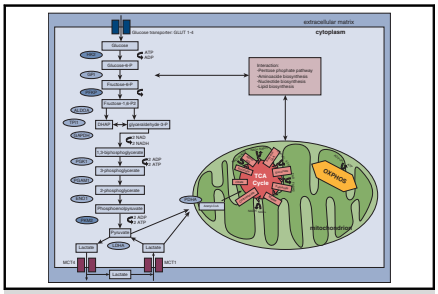
ABSTRACT

Objectives: Reprogramming of energy metabolism is a well-established hallmark of cancer, with aerobic glycolysis classically considered a prominent feature. We investigate the heterogeneity in glucose metabolism pathways within resectable primary lung adenocarcinoma and its clinical significance.

Methods: Using The Cancer Genome Atlas data, RNA expressions were extracted from 489 primary lung adenocarcinoma samples. Prognostic influence of glycolytic, aerobic, and mitochondrial markers (monocarboxylate transporter [*MCT*]*4*, *MCT1*, and translocase of outer mitochondrial membrane 20, respectively) was assessed using Kaplan-Meier analysis. Clustering of 35 genes involved in glucose metabolism was performed using the k-means method. The clusters were then analyzed for associations with demographic, clinical, and pathologic variables. Overall survival was assessed using the Kaplan-Meier estimator. Multivariate analysis was performed to assess the independent prognostic value of cluster membership.

Results: Classical statistical approach showed that higher expression of *MCT4* was associated with a significantly worse prognosis. Increased expression of translocase of outer mitochondrial membrane 20 was associated with a nonsignificant trend toward better prognosis, and increased expression of *MCT1* was associated with a better outcome. Clustering identified 3 major metabolic phenotypes, dominantly hypometabolic, dominantly oxidative, and dominantly mixed oxidative/glycolytic with significantly different pathologic stage distribution and prognosis; mixed oxidative/glycolytic was associated with worse survival. Cluster membership was independently associated with survival.

Conclusions: This study demonstrates the existence of distinct glucose metabolism clusters in resectable lung adenocarcinoma, providing valuable prognostic information. The findings highlight the potential relevance of considering metabolic profiles when designing strategies for reprogramming energy metabolism. Further studies are warranted to validate these findings in different cancer types and populations. (JTCVS Open 2024;20:194-201)



Summary of the 10 steps of glycolysis and interaction with the TCA cycle and OXPHOS.

CENTRAL MESSAGE

Using a clustering gene transcriptoma approach, we showed the existence of different energy metabolic phenotypes in lung adenocarcinoma and demonstrated that different clusters had different prognoses.

PERSPECTIVE

Most of the energy production of cancer cells originates from aerobic glycolysis. Concurrent tricarboxylic acid cycle and oxidative phosphorylation is a possible feature, but the clinical significance of the coexistence of both pathways is unknown. By a clustering approach, we showed the existence of different metabolic phenotypes and demonstrated that different clusters had different prognoses.

Reprogramming of energy metabolism has been recognized as a hallmark of cancer cells: increased energy demands are required to fuel cell growth and division.[1] In their landmark 2011 article, Hanahan and Weinberg[2] highlighted how aerobic glycolysis (the so-called Warburg effect) is largely favored over normal glucose metabolism in cancer cells:

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The Institutional Review Board did not approve this study, because of its nature (retrospective analysis of a publicly available database. Patient written consent for the publication of the study was not received, as they were anonymous.

Received for publication April 18, 2024; revisions received June 6, 2024; accepted for publication June 17, 2024; available ahead of print July 26, 2024.

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2666-2736

https://doi.org/10.1016/j.xjon.2024.06.010

Abbreviations and Acronyms

MCT = monocarboxylate transporter
MT-ND = mitochondrial NADH-ubiquinone oxidoreductase chain
OXPHOS = oxidative phosphorylation
PCA = principal component analysis
SLC16A1 = solute carrier family 16 member 1
SLC16A3 = solute carrier family 16 member 3
TCGA = The Cancer Genome Atlas
TOMM20 = translocase of outer mitochondrial membrane 20

Even under aerobic conditions, most of the energy production would come from cytoplasmic glycolysis, without the transfer of pyruvate to mitochondria for the tricarboxylic acid cycle and oxidative phosphorylation.

This mechanism, originally described by Otto Warbourg in 1930 and confirmed in various settings in the following years, is rather counterintuitive because the energetic efficiency of glycolysis (in terms of adenosine trisphosphate production) is about 18 times lower compared to the usual glucose metabolism of noncancerous cells.[3] To compensate for the lower energy efficiency and to enable enhanced glycolysis, cancer cells require more glucose to enter the cytosol, which is enabled by increased expression of glucose transporters, mainly GLUT1.[4,5] In this model, cancer can be viewed as a metabolic parasite whose cells compete for energy substrates with host cells in the rest of the body and in the tumor microenvironment.[6] The ability of tumors to take up glucose is clinically demonstrated using fludeoxyglucose F18 positron emission tomography, and a large body of literature has accumulated highlighting the negative prognostic character of increased glucose uptake.[7]

It should be emphasized that aerobic conditions are not necessarily present inside tumors whose neovascularization favors reduced oxygen availability, especially in the depth of the lesion; in this case, only glycolytic fueling allows cancer cells to survive and proliferate. In addition, glycolytic function has been shown to be associated with activated oncogenes such as RAS or MYC and mutated tumor suppressors such as *TP53*.[8-12] Furthermore, glycolytic waste in the tumor stroma contributes to a decrease in local pH, which in turn leads to a decrease in the efficacy of antitumor immune cells in the tumor microenvironment.[7,8]

Glycolysis is a sequence of 10 enzymatic reactions and is believed to have 3 key regulatory steps, namely the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase (first, third, and 10th steps, respectively). These regulatory steps are essentially irreversible and have large negative ΔG values. A large number of publications have been devoted to the influence of these enzymes on cancer progression and outcome, but most of the available literature focuses on a single enzyme, its expression and relationships with different oncogenic pathways.[13,14] However, it has been highlighted how several glycolytic intermediates, which are also generated in non-key steps and whose levels would be increased in the event of enhanced glycolysis and enhanced expression of non-key enzymes, can be diverted to enter various biosynthetic pathways, including those that generate nucleosides and amino acids, which in turn facilitate the biosynthesis of macromolecules required for cell division. Thus, upregulation of the whole glycolytic pathway would be a real advantage for cancer cells, mitigating the lower energy efficiency compared with normal glucose metabolism, but clinical evidence of increased aggressiveness in the case of current overactivation of the whole glycolytic pathway is currently poor.

Cancer cells do not necessarily rely on glycolysis, and Hanahan and Weinberg[2] highlighted that some tumors have been found to contain 2 subpopulations of cancer cells that differ in their energy production pathways: 1 subpopulation would consist of cells that rely on the Warburg effect and produce lactate, whereas the second subpopulation would preferentially import and use the lactate produced by their neighbors as their main energy source, converting lactate to pyruvate for use in the tricarboxylic acid cycle and oxidative phosphorylation. In this model, the role of tricarboxylic acid carriers is crucial, allowing lactic acid to be secreted or imported: The carriers responsible for this exchange, *MCT4* and *MCT1*, have been proposed to be more frequently associated with glycolytic or oxidative phosphorylation (OXPHOS) functions in cancer cells, respectively.[9,15,16] However, the clinical significance of the coexistence of both pathways has been poorly assessed.

Classifying tumor metabolism as oxidative, glycolytic, or both (if possible) on the basis of analysis of single or groups of proteins or gene expressions is not trivial. Indeed, it is not clear how one should classify the metabolism when, for example, some glycolytic genes show high RNA expression and others a low RNA expression. To our knowledge, there is no universally accepted heuristic for doing so. In this analysis, we propose to learn the classification heuristic on a publicly available dataset using a clustering approach.

Thus, using the RNA expressions available thanks to The Cancer Genome Atlas (TCGA) project and performing a clustering approach, we aimed to assess the coexistence of different glucose metabolism pathways in a clinical model of resectable primary lung adenocarcinoma; evaluate whether or not different clusters are differentially represented among stages, sex, and age categories; and assess the prognostic significance of different glucose metabolism pathway clusters.

MATERIAL AND METHODS

The R2 platform (http://r2.amc.nl) was used to extract demographic, pathologic, RNA expression, and overall survival data from the TCGA Lung Adenocarcinoma Database (Mixed Lung Adenocarcinoma (2022-v32) - tcga - 589 - tpm - gencode36). We took into account data on patients whose samples were represented by the primary tumor and for whom a minimal follow-up beyond intervention was available (n = 489). The institutional review board did not approve this study, because of its nature (retrospective analysis of a publicly available database). Patient written consent for the publication of the study was not received because they were anonymous.

First, we assessed the prognostic influence of RNA expression of a marker of glycolytic activity (ie, *MCT4*), a marker of aerobic activity (ie, *MCT1*), and a marker of mitochondrial function (ie, translocase of outer mitochondrial membrane 20 [TOMM20]) by Kaplan-Meier estimates of overall survival. We used the median, lower quartile, and upper quartile as cutoffs. Log-rank was used for comparison.

Secondly, we used the platform's *grabber* function to extract demographic and available clinical and pathological data. Together, we extracted RNA expression levels (normalized by log2) of 35 genes, a number considered compatible with the subsequent clustering as representing approximately 50% more than the square number of observations. Thus, we extracted the RNA expression of genes of the 3 main glucose transporters (solute carrier family 2 member 1 [SLC2A1], solute carrier family 2 member 3, and solute carrier family 2 member 4, whose proteins are known as GLUT1, GLUT3, and GLUT4), the 10 genes of glycolysis (hexokinase 2, glucose-6-phosphate isomerase, phosphofructokinase, aldoase fructose-bisphosphate A, triosephosphate isomerase 1, glyceraldehyde-3-phosphate, phosphoglycerate kinase 1, phosphoglycerate mutase 1, enolase 1, and pyruvate kinase M), the 8 genes of tricarboxylic cycle (citrase synthase, Aconitase 2, isocitrate dehydrogenase (NAD[+]) 3 catalytic subunit alpha, oxoglutarate dehydrogenase, succinate-CoA ligase GDP/ADP-forming subunit alpha, succinate dehydrogenase complex flavoprotein subunit A, fumarate hydratase, and malate dehydrogenase 2), and all 10 mitochondrial genes involved in oxidative phosphorylation (mitochondrially encoded ATP synthase membrane subunit 6, mitochondrially encoded ATP synthase membrane subunit 8, mitochondrially encoded cytochrome C oxidase I, mitochondrially encoded cytochrome C oxidase II, mitochondrially encoded cytochrome C oxidase III, mitochondrially encoded cytochrome B, mitochondrial NADH-ubiquinone oxidoreductase chain (MT-ND)1, MT-ND2, MT-ND3, MT-ND4) as well as of nonmitochondrial gene of OXPHOS, TOMM20, belonging to the TOMM complex. Finally, to take into account the possible utilization of lactate produced by glycolytic cancer cells by oxidative ones, we extracted the RNA expression of 2 tricarboxylic acid carrier genes, solute carrier family 16 member 3 (*SLC16A3*) and solute carrier family 16 member 1 (*SLC16A1*) (whose proteins are known as *MCT1* and *MCT4*), and of lactate dehydrogenase, which reversibly catalyze the pyruvate-lactate conversion.

For some of the enzymes different isoforms may exist; we selected, after careful literature review, the isoform more involved in glucose metabolism abnormalities in lung adenocarcinoma, whenever known, or, more generally, in cancer. For example, for hexokinase, we selected the isoform 2 (ie, hexokinase 2).

Data were retrieved in the txt format and converted to Excel (Microsoft Corporation). The Excel file was imported into Python and Python scripts were written to perform data analysis and clustering.

Clustering was performed using the k-means approach. Kaplan-Meier estimates were used to assess overall survival and curves were compared by log-rank test. Finally, Cox regression analysis was used to assess the independent character of prognostic factors. In the Cox model we included cluster, age, sex, and pathologic stage. Principal component analysis (PCA) analyses were performed to discuss the results qualitatively and display the relevant clusters. Used dataset and all the Python scripts are available upon request. Institutional review board review was not required due to the nature of the data analyzed.

RESULTS

Records of 489 patients who met our criteria were collected and available for analysis. All specimens were obtained at surgical resection for primary lung adenocarcinoma. The distribution of pathologic stages was I: 269, II: 116, III: 78, and IV: 25.

Prognostic Influence of Markers of Glycolytic or Oxidative Metabolism and of Mitochondrial Activity

Using median or 75th percentile but not the 25th percentile as cutoff, higher expression of SLC16A3 (*MCT4*, a marker of glycolytic metabolism) was associated with a significantly worse prognosis. Increased expression of SLC16A1 (*MCT1*, a marker of nonglycolytic metabolism) was associated with a better outcome when using the 75th percentile as cutoff, whereas the difference was not significant using the median or 25th percentile. Finally, increased expression of TOMM20 (a marker of OXPHOS), is associated with a trend toward better prognosis without reaching significance, regardless of the cutoff chosen (Figure 1).

Clustering Approach

We performed PCA on the gene expression variables to assist visualization of clusters. The cumulative explained variances for each principal component are shown in Figure 2, *A*. This result supports a 2-dimensional analysis of the clusters, as the first 2 components explain more than half of the variance in the dataset. Subsequent components explain far less variance. These 2 components correlate with each gene as shown in Figure 2, *B*.

We identified 4 clusters. On these 2 axes, the clusters can be visualized with a color mapping in Figure 2, *C*. Table 1 shows the mean ± SD of each analyzed gene expression as well as the means or proportions of available clinical variables in each cluster.

In a univariate Kaplan-Meier analysis, the assigned cluster is associated with overall survival (cluster 1 vs cluster 2 [$P < .05$], cluster 0 vs cluster 2 [$P = .01$], and cluster 1 vs cluster 0 [$P = .24$]). Cluster 3 was excluded from this analysis due to the small number of data points, and therefore large CIs. This can be seen in Figure 3. In a univariate Cox regression model (Table 2), clusters 0 and 1 are significantly associated with better prognosis compared with cluster 2, in the direction that is predicted by the Kaplan-Meier estimates. When age, sex and stage were included in the regression (Table 2), cluster 1 maintained significance, whereas cluster 0 lost significance, but a trend in the same direction was maintained.

DISCUSSION

In this study, we provide evidence that, in lung adenocarcinoma, genes of the entire glucose metabolism pathway (including glucose transporters, tricarboxylic acid

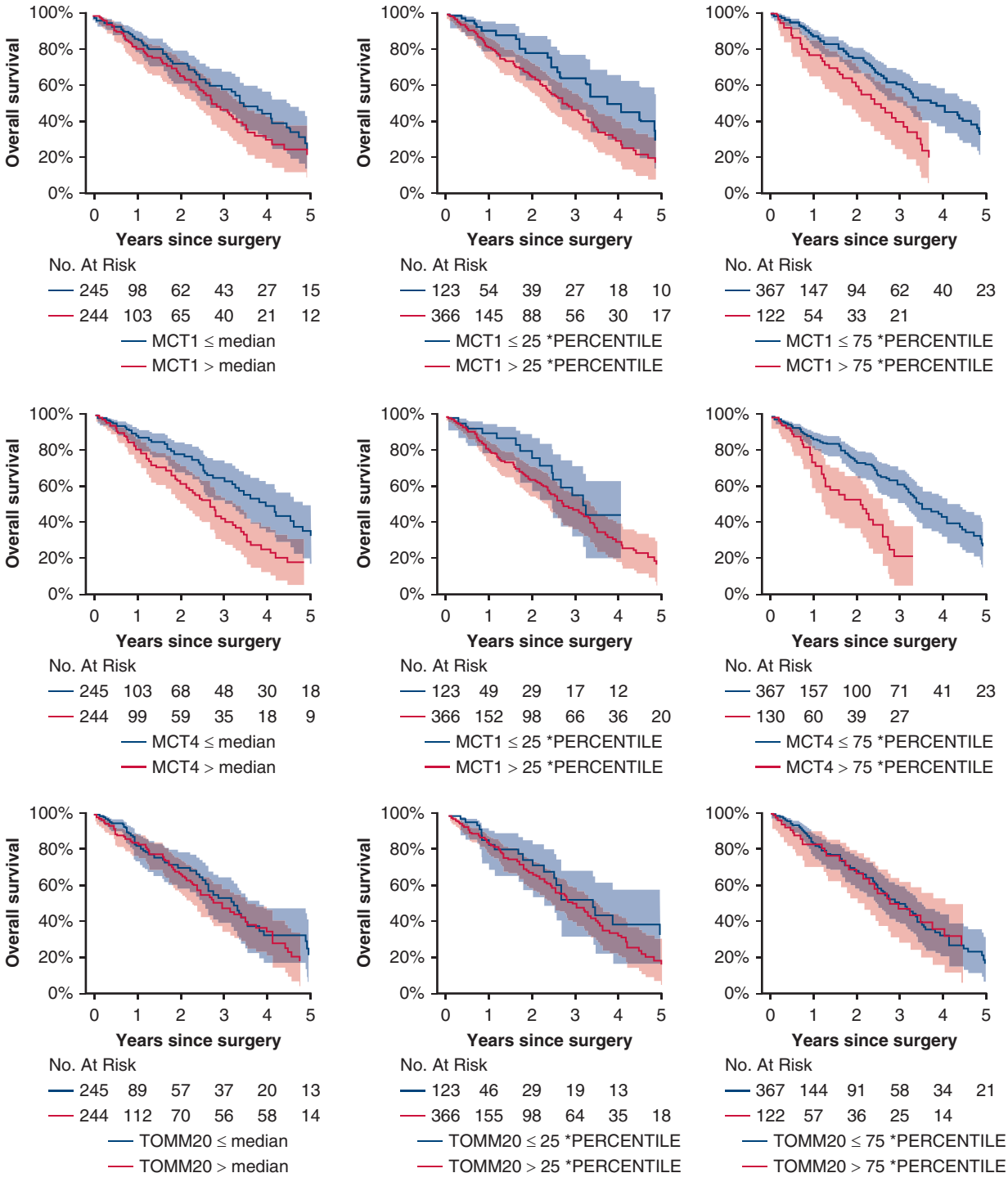

FIGURE 1. Prognostic influence of monocarboxylte transporter 4 (*MCT4*), monocarboxylate transporter 1 (*MCT1*), translocase of outer mitochondrial membrane 20 (*TOMM20*) using median, 75th and 25th percentile as cutoffs. Kaplan-Meier estimates of overall survival. Shadows indicate 95% CI.

transporters, glycolysis, tricarboxylic acid cycle, and OXPHOS) can be clustered, resulting in a strong prognostic discrimination. The prognostic significance of clusters is maintained in multivariate analysis underlining the independent prognostic significance.

This clustering approach has the advantage of not requiring labeled data; as discussed above, the labeling itself (ie, defining a tumor as merely glycolytic or not) may be hardly achievable. The expression of SLC16A3 (included in our analysis) has been reported as a general

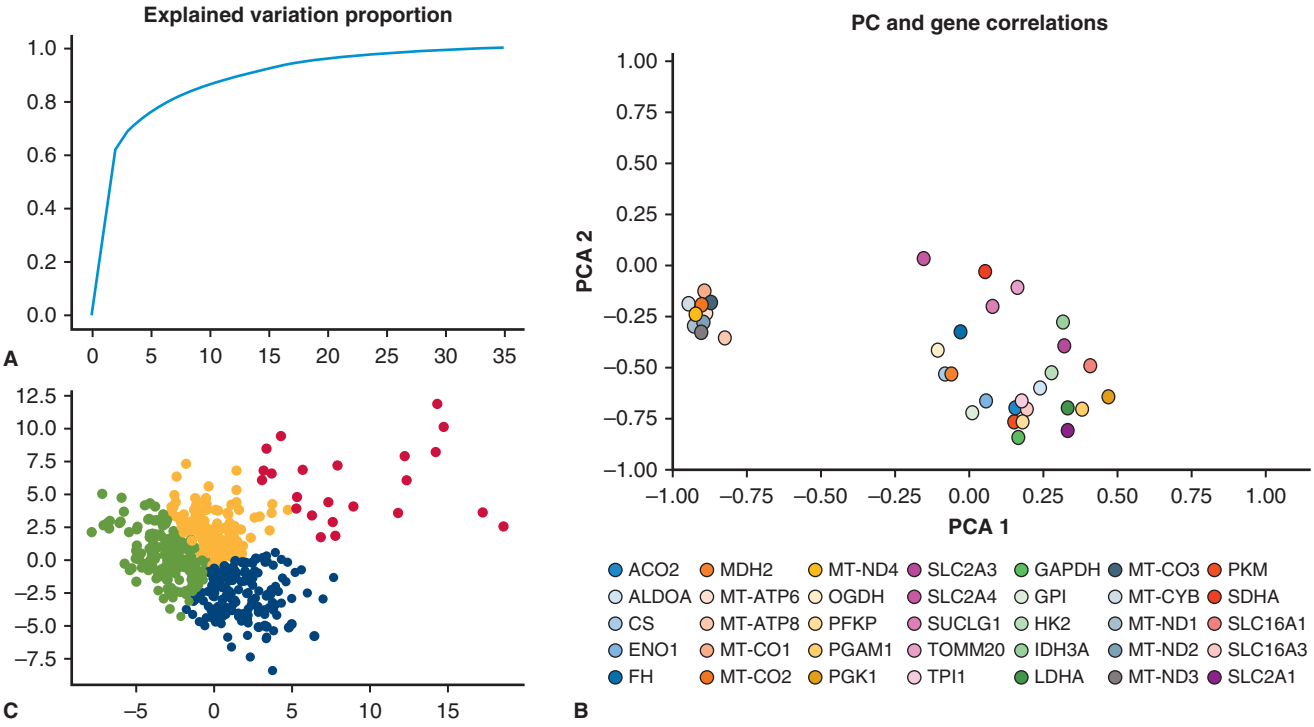

FIGURE 2. A, Explained variation proportion on gene expression used for clustering. B, Correlation between gene expression and principal components analysis (*PCA*). C, Representation of the 4 clusters: *blue* = cluster 0, *orange* = cluster 1, *green* = cluster 2, and *red* = cluster 3.

marker of glycolysis, because its product, *MCT4* is a tricarboxylic acid carrier responsible for lactate export from glycolytic-producing cells.[9,15-18] By a conventional statistical approach, using median as the cutoff, higher expression of SLC16A3, was associated with a significantly worse prognosis, compared with tumors with lower expression of *MCT4*, which could be considered nonglycolytic, although they cannot be directly qualified as relying on oxidative metabolism. We also show by a conventional approach that increased expression of TOMM20, classically considered as a marker of XPHOS, is associated with trend toward better prognosis without reaching significance (regardless from the cutoff chosen). Yet, increased expression of SLC16A1 (considered as marker of nonglycolytic metabolism) was associated with a better outcome when the 75th percentile was used as a cutoff, whereas the difference was not significant at median. The product of SLC16A1, *MCT1*, is a tricarboxylic acid carrier that uptake lactate from the interstitium (where it is rejected by glycolytic cells) into the nonglycolytic cells where it can be transformed into pyruvate by lactate dehydrogenase A.[9] The fate of pyruvate can be dual because it can proceed toward the tricarboxylic acid cycle and oxidative phosphorylation or even enter in the gluconeogenesis pathway to produce glucose, as in the Cori cycle. Using a conventional approach on TCGA data, one could conclude that glycolytic tumors have a worse prognosis as compared with nonglycolytic tumors and that tumors expressing a specific marker of OXPHOS show a nonsignificant trend toward better prognosis.

However, it should be underlined that most of the variance in gene expressions can be explained by 2-component PCA. In Figure 2, the x-axis is correlated with mitochondrial genes and therefore explains the aerobic character of the phenotype; the y-axis is correlated to anaerobic genes, but also with gene expressions related to lactate reuptake and lactate to pyruvate transformations. The fact that these 2 variables explain most of the variance in the dataset is consistent with basic research: these lung adenocarcinoma phenotypes are explained by a theoretical oxidative/mitochondrial variable, and a theoretical anaerobic variable. This second variable correlates with lactate and pyruvate metabolism, as predicted by the 2-components model.

Using our approach, we were able to identify 3 main clusters, including 171, 165, and 130 patients, respectively, and a smaller cluster with 23 individuals. The latter was mainly composed of younger individuals, with lower average tobacco consumption and more frequent early stage disease: expression of both oxidative and glycolytic genes was very low and few events were observed during the first years after surgery. The other 3 clusters were represented by a cluster (No. 1) expressing low levels of both glycolytic and OXPHOS genes, a cluster mainly relying on OXPHOS metabolism (No. 0) and a cluster (No. 2) with increased

TABLE 1. Clinical characteristics of each cluster and mean RNA expression levels

Feature	Cluster 0	Cluster 1	Cluster 2	Cluster 3
Mean age (y)	64.69 ± 10.31	67.38 ± 8.94	64.7 ± 10.1	57.96 ± 10.96
Women	56	58	50	43
Stage I	57	65	42	74
Stage II	20	22	29	17
Stage III	18	8	21	9
Stage IV	4	4	8	0
Pack-years	42.52 ± 28.34	38.8 ± 25.59	45.35 ± 29.11	30.67 ± 17.17
ACO2	4.02 ± 0.56	4.29 ± 0.68	4.47 ± 0.53	3.4 ± 0.52
ALDOA	2.68 ± 0.75	2.58 ± 0.59	3.49 ± 0.72	2.46 ± 0.99
CS	6.09 ± 0.5	6.12 ± 0.52	6.56 ± 0.52	5.18 ± 0.66
ENO1	9.87 ± 0.64	9.74 ± 0.73	10.64 ± 0.6	8.82 ± 1.24
FH	6.42 ± 0.51	6.54 ± 0.59	6.74 ± 0.5	5.78 ± 0.57
GAPDH	10.18 ± 0.66	9.95 ± 0.72	11.32 ± 0.6	9.25 ± 1.29
GPI	6.26 ± 0.58	6.04 ± 0.54	6.96 ± 0.6	5.23 ± 0.7
HK2	4.88 ± 1.03	4.56 ± 1.08	5.95 ± 0.98	4.96 ± 1.36
IDH3A	2.72 ± 0.59	3.04 ± 0.46	3.26 ± 0.52	3.05 ± 0.5
LDHA	8.31 ± 0.62	8.34 ± 0.57	9.31 ± 0.65	8.27 ± 0.68
MDH2	7.32 ± 0.57	7.31 ± 0.63	7.76 ± 0.51	6.33 ± 0.7
MT-ATP6	13.89 ± 0.48	12.69 ± 0.56	12.8 3 ± 0.69	9.36 ± 1.95
MT-ATP8	11.69 ± 0.76	10.72 ± 0.74	10.89 ± 0.73	7.04 ± 1.78
MT-CO1	14.82 ± 0.49	13.73 ± 0.48	13.71 ± 0.69	11.63 ± 1.25
MT-CO2	14.71 ± 0.49	13.47 ± 0.5	13.65 ± 0.66	11.06 ± 1.2
MT-CO3	14.79 ± 0.52	13.53 ± 0.5	13.67 ± 0.71	11.56 ± 1.13
MT-CYB	13.84 ± 0.52	12.64 ± 0.53	12.59 ± 0.71	9.76 ± 1.9
MT-ND1	13.73 ± 0.57	12.55 ± 0.58	12.51 ± 0.83	9.04 ± 1.96
MT-ND2	13.78 ± 0.58	12.48 ± 0.57	12.64 ± 0.71	8.58 ± 2.28
MT-ND3	13.53 ± 0.56	12.63 ± 0.56	12.63 ± 0.62	9.96 ± 1.12
MT-ND4	14.63 ± 0.47	13.39 ± 0.53	13.52 ± 0.71	9.86 ± 2.11
OGDH	6.45 ± 0.62	6.33 ± 0.59	6.68 ± 0.57	5.47 ± 0.75
PFKP	5.32 ± 0.99	4.81 ± 1	6.36 ± 0.96	4.66 ± 0.94
PGAM1	5.22 ± 0.57	5.41 ± 0.46	6.22 ± 0.52	5.14 ± 0.68
PGK1	7.91 ± 0.7	8.12 ± 0.59	9.09 ± 0.63	8.19 ± 0.96
PKM	8.59 ± 0.59	8.59 ± 0.65	9.47 ± 0.5	7.66 ± 0.87
SDHA	1.81 ± 0.48	1.69 ± 0.43	1.88 ± 0.5	2.29 ± 0.93
SLC16A1	2.84 ± 1.08	2.85 ± 0.99	4.37 ± 1.44	3.82 ± 0.94
SLC16A3	5.13 ± 1.02	4.88 ± 0.87	6.21 ± 0.92	4.42 ± 1.2
SLC2A1	5.32 ± 1.21	4.73 ± 0.97	7.17 ± 1.06	5.24 ± 1.28
SLC2A3	4.36 ± 1.1	4.4 ± 1.12	5.34 ± 1.21	4.72 ± 1.22
SLC2A4	0.72 ± 0.48	0.65 ± 0.37	0.62 ± 0.52	0.47 ± 0.32
SUCLG1	5.48 ± 0.42	5.68 ± 0.53	5.78 ± 0.47	5.18 ± 0.75

(Continued)

TABLE 1. Continued

Feature	Cluster 0	Cluster 1	Cluster 2	Cluster 3
TOMM20	7.57 ± 0.61	8.01 ± 0.65	7.96 ± 0.51	7.63 ± 0.35
TPI1	8.97 ± 0.51	8.86 ± 0.69	9.88 ± 0.53	8.15 ± 1.24

Values are presented as mean ± SD or %. *ACO2*, Aconitase 2; *ALDOA*, aldolase, fructose-bisphosphate A; *CS*, citrate synthase; *ENO1*, enolase 1; *FH*, fumarate hydratase; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *GPI*, glucose-6-phosphate isomerase; *HK2*, hexokinase 2; *IDH3A*, isocitrate dehydrogenase (NAD(+)) 3 catalytic subunit alpha; *LDHA*, lactate dehydrogenase A; *MDH2*, malate dehydrogenase 2; *MT-ATP6*, mitochondrially encoded ATP synthase membrane subunit 6; *MT-ATP8*, mitochondrially encoded ATP synthase membrane subunit 8; *MT-CO1*, mitochondrially encoded cytochrome C oxidase I; *MT-CO2*, mitochondrially encoded cytochrome C oxidase II; *MT-CO3*, mitochondrially encoded cytochrome C oxidase III; *MT-CYB*, mitochondrially encoded cytochrome B; *MT-ND1*, mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 1; *MT-ND2*, mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 2; *MT*-ND3, mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 1; *MT-ND4*, mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 4; *OGDH*, oxoglutarate dehydrogenase; *PFKP*, phosphofructokinase, platelet; *PGAM1*, phosphoglycerate mutase 1; PGK1, phosphoglycerate kinase 1; *PKM*, pyruvate kinase M; *SDHA*, succinate dehydrogenase complex flavoprotein subunit A; *SLC16A1*, solute carrier family 16 member 1; *SLC16A3*, solute carrier family 16 member 3; *SLC2A1*, solute carrier family 2 member 1; *SLC2A3*, solute carrier family 2 member 3; *SLC2A4*, solute carrier family 2 member 4; *SUCLG1*, succinate-CoA ligase GDP/ADP-forming subunit alpha; *TOMM20*, translocase of outer mitochondrial membrane 20; *TPI1*, triosephosphate isomerase 1.

levels of expression of glycolytic genes, but also expressing genes responsible for lactate reuptake and transformation to pyruvate at a higher level compared with clusters 0 and 1, and expressing also mitochondrial genes at a level on average superior to the hypometabolic cluster, but similar to the cluster relying mainly on OXPHOS. The outcome was different among the 3 clusters, with the best survival in the cluster (No. 1) with low expression of both oxidative and glycolytic genes, followed by the cluster with dominant OXPHOS metabolism (No. 0), and finally by the cluster (No. 2) with glycolytic metabolism associated with OXPHOS 1. Notably, this prognostic significance applies to the entire population, but similar trends are observed within the different stages of the disease. In other words, our findings support the concept that inside a tumor harboring a glycolytic component the presence of a nonglycolytic

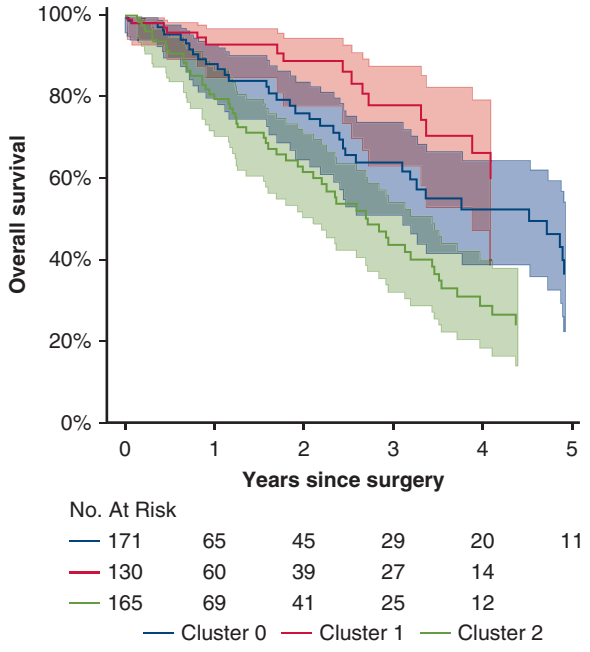

FIGURE 3. Kaplan-Meier estimates of overall survival by clusters. Shadows indicate 95% CI.

1 conjure to increase aggressiveness, which is reflected by worsening prognosis.

Thus, the results of our approach seem to be consistent with some knowledge from basic science and show that clustering could allow the identification of metabolic phenotypes associated with different prognoses in clinical settings.[19-21] This could be potentially relevant on clinical grounds, and could support the concept that reprogramming tumor metabolism should be a major research goal in coming years.[2,22,23] In the era of personalized medicine, allocating patients to a specific group of metabolic profiles would be crucial in the design of such studies on energy metabolism reprogramming strategies.[24]

Previous studies based on TCGA data have shown the enormous potential of this project to reveal multiple metabolic pathway dysregulation in different cancers.[25] In this study we focused on a specific cancer type and demonstrated the existence of different clusters with a strong influence on survival: We believe that the main strength of our study is the relative simplicity of the concepts. There are 2 main limitations of our study. The first is due to the nature of the samples: The RNA was extracted from bulk tumors and therefore contained variable amounts of host RNA along with the tumor RNA, which is likely to represent the dominant part of the RNA: Despite an unavoidable limitation, the bulk approach has the advantage of taking into account tumor heterogeneity, which cannot be easily taken into account when using other approaches, such as single-cell RNA analysis. The other limitation is that the results of our method should be considered as dataset specific. Unfortunately, to date, publicly available transcriptome datasets (eg, lung squamous cell - China or lung squamous cell - Korea[26]) contain a limited number of patients, which precludes a clustering approach.

Overall, patterns and correlations derived from the TCGA dataset we used are specific to the cancer type studied in this article; that is, primary lung adenocarcinoma. The behavior (including influence on survival) in other histologic types of lung cancer and, more generally, in other cancers and relative comparisons deserve further study.

TABLE 2. Univariate and multivariate Cox regression for each cluster*

Feature	coef	exp(coef)	se(coef)	coef lower 95%	coef upper 95%	exp(coef) lower 95%	exp(coef) upper 95%	*z*	*P* value	−log2(p)
Univariate										
Cluster 0	−0.54	0.58	0.21	−0.95	−0.14	0.39	0.87	−2.63	.01	6.85
Cluster 1	−0.87	0.42	0.25	−1.36	−0.37	0.26	0.69	−3.44	<.005	10.73
Multivariate										
Cluster 0	−0.28	0.76	0.21	−0.69	0.14	0.50	1.15	−1.31	.19	2.40
Cluster 1	−0.80	0.45	0.26	−1.31	−0.28	0.27	0.76	−3.00	<.005	8.54
Sex	0.17	1.18	0.19	−0.20	0.53	0.82	1.70	0.88	.38	1.41
Age	0.02	1.02	0.01	0.01	0.04	1.01	1.04	2.51	.01	6.36
Stage II	0.94	2.55	0.24	0.47	1.40	1.60	4.06	3.94	<.005	13.57
Stage III	1.34	3.81	0.24	0.87	1.81	2.39	6.08	5.62	<.005	25.65
Stage IV	1.34	3.83	0.36	0.64	2.05	1.90	7.74	3.75	<.005	12.45

coef, Coefficients; *exp(coef)*, hazard ratios, obtained by exponentiation the coefficients; *se(coef)*, standard error of the coefficients; *−log2(p)*, negative logarithm in base two of the *P* value. *Cluster 2 and stage I are the reference categories.

Conflict of Interest Statement

The authors reported no conflicts of interest.

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Key Words: glucose metabolism, gene clustering, lung cancer, survival