Loss of the Mitochondrial Bioenergetic Capacity Underlies the Glucose Avidity of Carcinomas

Fernando López-Ríos,[1] María Sánchez-Aragó,[2] Elena García-García,[1] Álvaro D. Ortega,[2] José R. Berrendero,[3] Francisco Pozo-Rodríguez,[1] Ángel López-Encuentra,[1] Claudio Ballestín,[1] and José M. Cuezva[2]

[1]Hospital Universitario 12 de Octubre, CIBER de Enfermedades Respiratorias; [2]Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa, CIBER de Enfermedades Raras, Universidad Autónoma de Madrid-Consejo Superior de Investigaciones Cientificas; and [3]Departamento de Matemáticas, Universidad Autónoma de Madrid, Madrid, Spain

Abstract

The down-regulation of the catalytic subunit of the mitochondrial H^+-ATP synthase (β-F1-ATPase) is a hallmark of most human carcinomas. This characteristic of the cancer cell provides a proteomic signature of cellular bioenergetics that can predict the prognosis of colon, lung, and breast cancer patients. Here we show that the *in vivo* tumor glucose uptake of lung carcinomas, as assessed by positron emission tomography in 110 patients using 2-deoxy-2-[^{18}F]fluoro-D-glucose as probe, inversely correlates with the bioenergetic signature determined by immunohistochemical analysis in tumor surgical specimens. Further, we show that inhibition of the activity of oxidative phosphorylation by incubation of cancer cells with oligomycin triggers a rapid increase in their rates of aerobic glycolysis. Moreover, we show that the cellular expression level of the β-F1-ATPase protein of mitochondrial oxidative phosphorylation inversely correlates ($P < 0.001$) with the rates of aerobic glycolysis in cancer cells. The results highlight the relevance of the alteration of the bioenergetic function of mitochondria for glucose capture and consumption by aerobic glycolysis in carcinomas. [Cancer Res 2007;67(19):9013–7]

Introduction

The cellular uptake of the nonmetabolizable 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) used in positron emission tomography (PET) has emerged as a valuable tool for diagnosing and staging cancer patients (1). Tumor capture of FDG is expressed by the standardized uptake value (SUV), which provides an estimation of glucose consumption by tumor cells. The increased glucose capture of tumor cells could result from an augmented demand for carbon skeletons to sustain uncontrolled proliferation and/or from a shift in the pathway used for energy provision during cellular proliferation. The latter was suggested by Otto Warburg many years ago (2) but remained largely unexplored until the recent renaissance of the so-called "Warburg effect" in cancer biology (3).

It has been shown that most types of cancers (4–6) fit the Warburg hypothesis because a decreased expression of mitochondrial H^+-ATP synthase (β-F1-ATPase), which is a bottleneck of mitochondrial oxidative phosphorylation, is linked to an increased expression of several markers of the glycolytic pathway (4, 5). This proteomic feature of cancer was defined as "the bioenergetic signature" and shown to provide a relevant marker of disease progression in colon, lung, and breast cancer patients (4–6). Moreover, it has been predicted that the bioenergetic signature further provides a marker of the cellular response to chemotherapy (7).

In this work, we show that glucose uptake of lung carcinomas, as assessed *in vivo* by FDG-PET imaging in the clinical setting, correlates with proteomic markers of the bioenergetic signature determined in surgical specimens. Moreover, we show that the rate of aerobic glycolysis in cancer cells depends on the activity of oxidative phosphorylation and on the expression level of markers of the bioenergetic signature. These results support, in part, that the increased glucose avidity of carcinomas results from a shift in the pathways of energy provision in the cancer cell and thus support Warburg's hypothesis.

Materials and Methods

Patients. Surgical specimens, study procedures, and clinical information of patients with potentially respectable non–small-cell lung cancer were those described previously (8). A summary of relevant clinicopathologic information of the cohort of patients analyzed is provided in Table 1.

PET studies. PET examinations were done in 6-h-fasted patients using an ECAT EXACT 47 (Siemens-CTI) camera with whole-body attenuation (8). Patients entering the PET study had blood glucose concentrations <7 mmol/L. The transmission study (slices of 16.6-cm width) was done with a germanium-68 source during 20 min. After, the patient was injected an i.v. dose of 370 MBq of [^{18}F]FDG. The emission study commenced 45 min after the injection and lasted for 20 min. Slices of 16.2-cm width were subsequently obtained with a 5-min exposition each and corrected for attenuation with a Sun Ultra 60 computer 2 × 450 MHz with an iterative reconstruction-type, attenuation-weighted ordered-subsets expectation maximization. Measurements were conducted consistently in each patient. Regions of interest were manually drawn on the transaxial images around the most intense focal [^{18}F]FDG uptake zone in the primary tumor, and the maximum SUV (SUV_{max}) for each patient was used to minimize the partial volume effects. It should be noted that clinical decisions were blinded to PET results.

Immunohistochemistry. Tissue microarrays containing triplicate 1-mm cores from selected tumor areas of paraffin-embedded lung carcinomas were constructed. The primary antibodies used were anti–β-F1-ATPase (1:3,000; ref. 4), anti-Hsp60 (1:400; SPA 807, StressGen), anti–glyceraldehyde-3-phosphate dehydrogenase (GADPH; 1:8,000), and anti–pyruvate kinase (1:2,000; both from Abcam). The expression level of the markers was scored as previously described (ref. 4; see also Fig. 1).

Note: M. Sánchez-Aragó and E. García-García contributed equally to this work.

Present address for E. García-García: Laboratorio de Dianas Terapéuticas, Centro Integral Oncológico "Clara Campal," 28050 Madrid, Spain.

Requests for reprints: José M. Cuezva, Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, 28049 Madrid, Spain. Phone: 34-91-497-4866; Fax: 34-91-497-4799; E-mail: jmcuezva@cbm.uam.es.

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Table 1. Summary of clinicopathologic characteristics of the cohort of patients studied

Characteristics	
Age, y	
Range	43-82
Mean	65
Gender, *n* (%)	
Male	104 (96)
Female	6 (4)
Histology, *n* (%)	
SCC	63 (57)
AC	26 (24)
Others	21 (19)
T stage, *n* (%)	
T_1	25 (23)
T_2	65 (59)
T_3	12 (11)
T_4	8 (7)
N stage, *n* (%)	
N_0	65 (59)
N_1	17 (15)
N_2	23 (21)
N_3	5 (5)
M stage, *n* (%)	
M_0	93 (85)
M_1	17 (15)
SUV_{max}, *n* (%)	
≤5.0	40 (36)
>5.0	68 (62)
Unknown	2 (2)

Abbreviations: SCC, squamous cell carcinoma; AC, adenocarcinoma.

Cell cultures. Human liver (HepG2), lung (HOP62), and colon (KM12 and HCT116) cancer cells were grown. To change the bioenergetic phenotype of HCT116 cells (9), cells were incubated with 6 μmol/L oligomycin or 10 mmol/L 2-desoxiglucose for 2 days. The antibodies used for Western blotting were anti–β-F1-ATPase (1:20,000), anti-Hsp60 (1:2,000), anti-GADPH (1:20,000), and anti–pyruvate kinase (1:1,000). For the determination of aerobic glycolysis, cells were incubated with or without 6 μmol/L oligomycin to assess the relevance of oxidative phosphorylation in the rates of aerobic glycolysis. At various times, 0.1-mL aliquots of the culture media were collected and used for the enzymatic determination of lactate (10).

Statistical analysis. Pearson's and Spearman's coefficients were used for linear correlation studies. Simple and multiple linear regression models were fitted to the data. Response variables were SUV_{max} and SUV normalized for the tumor size (TSN-SUV). Explanatory variables were the expression levels of mitochondrial and glycolytic markers. In all cases, the global significance of the models and the individual significance of each explanatory variable were assessed using standard *F* test and Student's *t* test. One-way ANOVA was used to detect differences in SUV_{max} for different levels of β-F1-ATPase/GAPDH ratio in the tumors. Standard *F* tests were used to assess significance. To determine the degree of association of SUV_{max} and β-F1-ATPase expression with survival, the mean values of both variables were used as cutoff points to define potentially "high-risk" and "low-risk" groups. Survival curves were computed using Kaplan-Meier estimates and compared using the log-rank test. Cox proportional hazards regression methods were used for multivariate survival analysis.

Results

A set of 110 lung carcinomas with full clinical annotation including PET data were studied (Table 1; ref. 8). Tissue sections were reviewed by two pathologists to reclassify lung tumors according to the 2004 WHO Classification and processed for immunohistochemical analysis of mitochondrial (β-F1-ATPase and Hsp60) and glycolytic (GAPDH and pyruvate kinase) markers of the bioenergetic signature of the cell (Fig. 1; refs. 4, 5). The FDG uptake of the tumor assessed by the SUV_{max} was also normalized for the tumor size (TSN-SUV) to obtain an estimation of the avidity for glucose per tumor unit, using the largest diameter (in centimeters) of the surgical specimen as determined by the pathologist.

We observed that in our cohort of patients, a lower SUV_{max} of the tumor was a significant prognostic factor of survival (Fig. 2*A*), consistent with recent findings (11). A direct linear correlation was observed between tumor SUV_{max} data and the expression level of GAPDH ($R = 0.196$, $P = 0.044$), suggesting that glucose capture by the tumor is linked to its rate of utilization

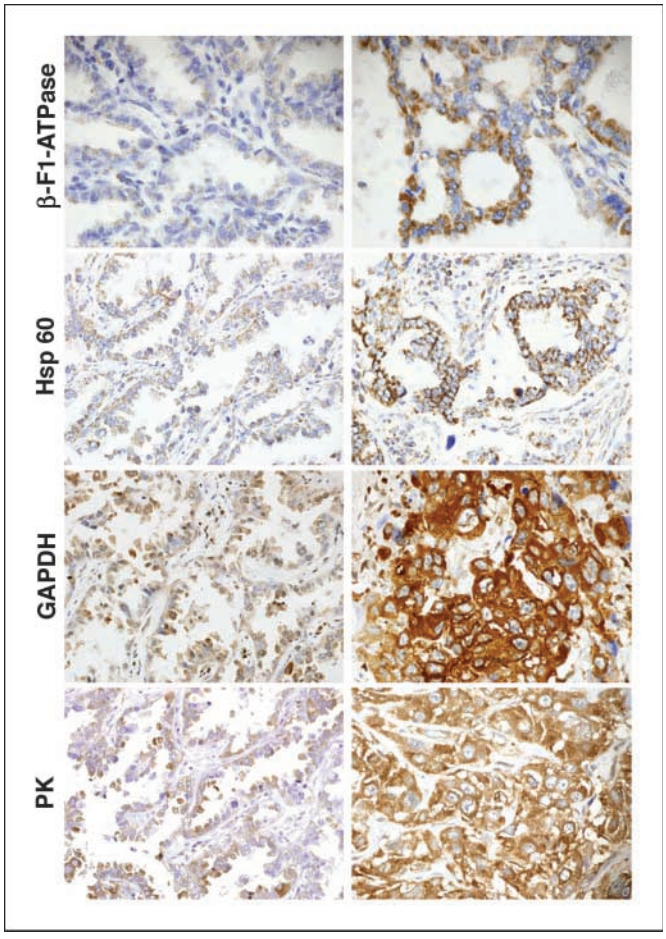

Figure 1. Expression of markers of the bioenergetic signature in lung carcinomas. Tissue microarray sections were cut and processed for immunohistochemical analysis of the markers of the bioenergetic signature. Representative examples of low (*left*) and high (*right*) expression levels of the mitochondrial β-F1-ATPase and Hsp60 as well as of the glycolytic GAPDH and pyruvate kinase (*PK*) markers. Note the preferential granular perinuclear staining of the cytoplasm revealing mitochondria in β-F1-ATPase and Hsp60 and the diffuse staining of the cytoplasm in GAPDH and pyruvate kinase.

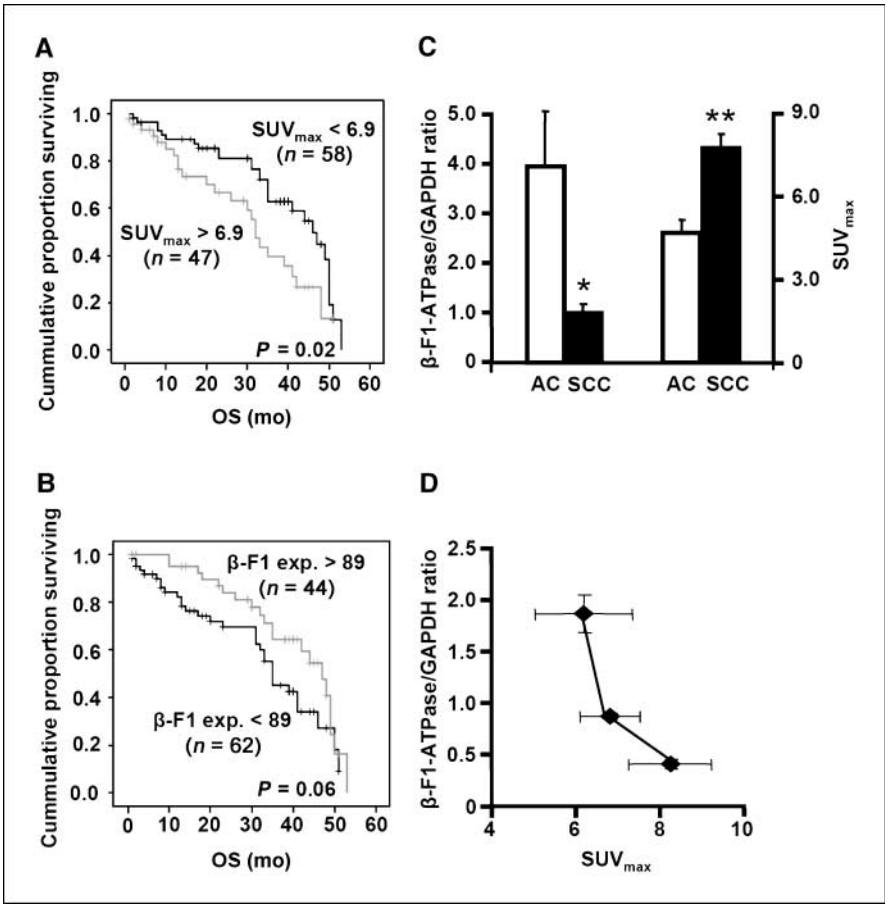

Figure 2. Tumor glucose uptake and the bioenergetic signature of non–small-cell lung cancer. *A* and *B,* Kaplan-Meier survival analysis shows the association of FDG uptake (SUV_{max}) and β-F1-ATPase expression in lung cancer with patients' overall survival (*OS*), respectively. *C,* the bioenergetic signature (β-F1-ATPase/GAPDH ratio) and FDG uptake (SUV_{max}) of adenocarcinomas (*AC*) and squamous cell carcinomas (*SCC*) of the lung. *Columns,* mean of 26 and 63 tumors for adenocarcinoma and squamous cell carcinoma, respectively; *bars,* SE. *, $P < 0.05$; **, $P < 0.01$, compared with adenocarcinoma. *D,* the bioenergetic signature of lung carcinomas defines the rates of glucose uptake (SUV_{max}) as assessed by FDG-PET. *Points,* mean of 33/32, 38/37, and 37/37 tumors for β-F1-ATPase/GAPDH ratio and SUV_{max} data, respectively; *bars,* SE. The relationship between the two variables is significant ($P = 0.048$) as assessed by standard *F* test in a one-way ANOVA model.

by glycolysis. Although no correlation was observed between tumor SUV_{max} data and pyruvate kinase expression, both glycolytic markers (pyruvate kinase and GAPDH) significantly correlated in the surgical specimens ($R = 0.368$, $P < 0.001$), supporting a concerted adaptation of lung tumors to a glycolytic phenotype (4, 5). A significant inverse correlation was found between TSN-SUV values and the expression level of β-F1-ATPase in the tumors ($R = -0.308$, $P = 0.004$). In contrast with this finding, no correlation was observed between the expression of Hsp60, a structural marker of mitochondria, and SUV_{max} or TSN-SUV data, suggesting that the loss of the bioenergetic marker of mitochondria underlies the increased glucose uptake of the cancer cell. Multiple regression analysis supports that tumor size ($P = 0.001$) and β-F1-ATPase expression ($P = 0.007$) are independent explanatory variables for TSN-SUV. These linear correlations also hold when patients with advanced disease were excluded from the analysis. In agreement with previous findings (6), it was found that patients with a higher tumor expression level of β-F1-ATPase had a higher life expectancy (Fig. 2*B*), whereas other markers provided no meaningful correlations with survival. Moreover, multivariate Cox regression analysis indicated that β-F1-ATPase expression ($P = 0.044$), SUV_{max} ($P = 0.020$), and tumor stage ($P = 0.010$) are independent markers of survival, although these correlations did not reach the level of significance when applied to the cohort of patients that underwent surgery, most likely because of the reduction in the number of cases.

The β-F1-ATPase/GAPDH ratio is a proteomic index of the overall mitochondrial capacity of the cell (4–6). It was found that squamous cell carcinomas displayed a lower β-F1-ATPase/GAPDH ratio than lung adenocarcinomas (Fig. 2*C*) and had significantly higher SUV_{max} values (Fig. 2*C*). Moreover, the stratification of lung carcinomas based on the overall mitochondrial capacity of the cell also significantly related ($P = 0.048$) with the rates of glucose consumption as assessed by FDG capture (Fig. 2*D*).

To illustrate the relevance of oxidative phosphorylation in the rate of glucose consumption by aerobic glycolysis, various human cancer cell lines were treated with oligomycin, an inhibitor of H^+-ATP synthase. Oligomycin treatment promoted a rapid burst in glucose consumption in the cells as assessed by the rapid increase in their rates of aerobic glycolysis (Fig. 3*A*). Likewise, the regulation of the overall mitochondrial activity of HCT116 cells (9), which results in cells expressing different levels of β-F1-ATPase (Fig. 3*B*), also showed a significant inverse relationship ($P < 0.001$) between the rate of glucose consumption by aerobic glycolysis and the mitochondrial capacity of the cell (Fig. 3*B*). Taken together, these results highlight both *in vivo* and *in vitro* the relevance of the bioenergetic function of mitochondria for cellular glucose uptake and consumption.

Discussion

The onset of the Warburg effect in the cancer cell (i.e., the shift to a glycolytic phenotype) has been explained on the grounds of

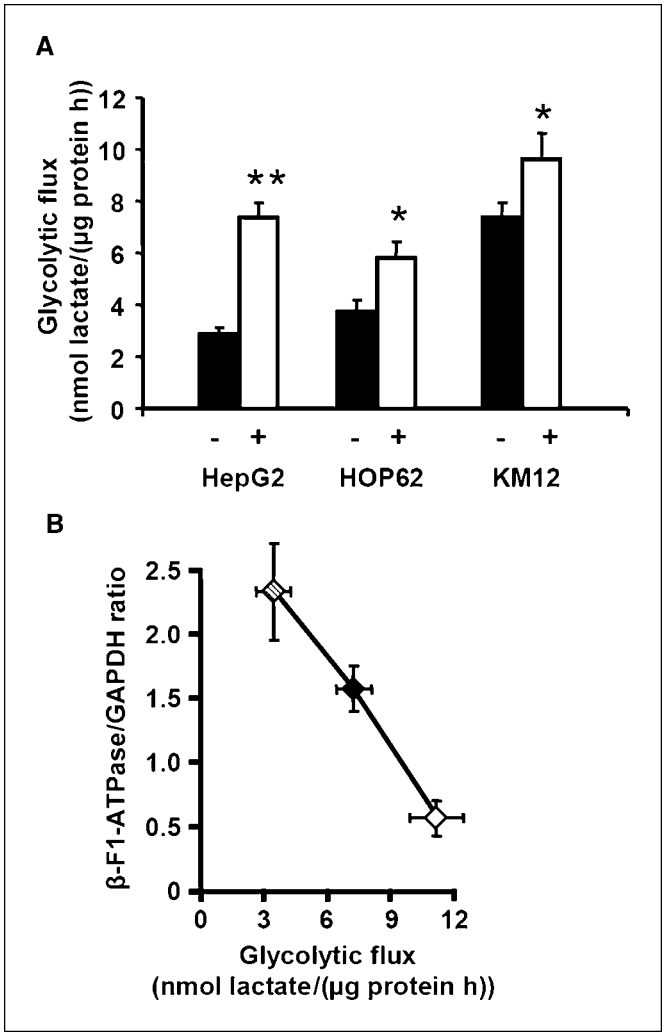

Figure 3. The activity of oxidative phosphorylation and the bioenergetic signature of the cell define the rate of glucose consumption by aerobic glycolysis. *A,* the flux of aerobic glycolysis of cancer cells (HepG2, HOP62, and KM12) increases when the activity of the mitochondrial H^+-ATPase is inhibited by incubation of the cells with oligomycin (+). *Columns,* mean of three to six determinations; *bars,* SE. *, $P < 0.05$; **, $P < 0.005$, compared with nontreated (−) cells. *B,* the bioenergetic signature (β-F1-ATPase/GAPDH ratio) of HCT116 cells defines the rates of glucose utilization by aerobic glycolysis. HCT116 cells were incubated with 10 mmol/L 2-desoxiglucose (*hatched symbol*) or 6 μmol/L oligomycin (*open symbol*), or left untreated (*closed symbol*), for 48 h to promote changes in the cellular bioenergetic phenotype. After removal of the drugs, the cells were maintained in culture for the determination of the bioenergetic signature and rates of glycolysis. *Points,* mean of 4 (*hatched*), 9 (*closed*), and 5 (*open*) independent determinations for β-F1-ATPase/GAPDH ratio and of 6 (*hatched*), 15 (*closed*), and 8 (*open*) determinations for glycolytic flux; *bars,* SE. The relationship between the two variables is highly significant ($P < 0.001$) as assessed by standard F test in a one-way ANOVA model.

(*a*) metabolic adaptation to the hypoxic environment where the tumor develops (12) and/or by a direct effect of hypoxia-inducible factor 1α on mitochondrial bioenergetics (13); (*b*) mutations in oncogenes and proteins related to signal transduction pathways (myc, Akt, and mTOR) that, in turn, promote changes in the expression of genes involved in cellular energetic metabolism (10, 14) or directly interfere with mitochondrial bioenergetics by affecting the biogenesis of specific respiratory complexes (15); and finally, (*c*) by mutations in mitochondrial DNA (16) or in nuclear genes involved in the metabolic and bioenergetic function of the organelle (17). However, superimposed to any of these genetic and/or epigenetic alterations, it is possible that the shift to a glycolytic phenotype is hardwired in cancer cells because glycolysis is the metabolic pathway required for cellular proliferation. In fact, it has been described that lymphocytes shift to glycolysis during cellular proliferation (18). Moreover, progression through the cell cycle is supported by nonrespiratory modes of energy generation as a result of the inhibition of mitochondrial function by cyclin D1 (19). In this regard, the late cell cycle biosynthesis of β-F1-ATPase, which mostly occurs at G_2-M by a mechanism that requires the internal ribosome entry site translational activity of the 3′ untranslated region of the transcript (20), could suggest that differences in the bioenergetic signature of tumor specimens might also arise as a result of diverse rates of cellular proliferation in the tumor.

Overall, we provide for the first time a link between the expression of a mitochondrial protein involved in energy transduction (β-F1-ATPase) and the functional estimation of the rate of glucose capture and consumption in cancer cells. The results support that an altered oxidative phosphorylation is one of the determinants that underlies the abnormal aerobic glycolysis of the cancer cell, sustaining Warburg's hypothesis and affording a mechanistic explanation for FDG-PET imaging in oncology.

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