Relationship between transcriptional expression of pyruvate dehydrogenase and local control of disease in patients with oral cavity carcinomas

Relación entre la expresión transcripcional de piruvato deshidrogenasa y el control local de la enfermedad en pacientes con cáncer en la cavidad oral

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Abstract

Background

The altered cellular metabolism is one of the hallmarks of the cancer cells, favoring the process of aerobic glycolysis, known as the Warburg effect. The pyruvate dehydrogenase (PDH) complex is one of the elements involved in this metabolic process. The present study aims to evaluate the relationship between the transcriptional expression of PDHB and the risk of local recurrence in patients with oral cavity carcinomas.

Methods

We determined the transcriptional expression of PDHB in biopsies from 41 patients with oral cavity carcinomas treated with surgery. The PDHB expression was categorized according to the local control of the disease with a recursive partitioning analysis.

Results

During the follow-up period 13 patients (31.7%) had a local recurrence of the tumor. Considering local disease control as the dependent variable, the recursive partitioning analysis classified the patients in two categories according to high (n = 16, 39.0%) or low (n = 25, 61.0%) PDHB expression. Five-year local recurrence-free survival for patients with high PDHB expression was 84.8% (95% CI: 65.2-100%), and for patients with low expression it was 54.3% (95% CI: 34.3–74.2 %) (P = 0.034). The results of multivariate analysis showed that patients

with a low PDHB expression had a 4.90 times higher risk of local recurrence of the tumor (95% CI: 1.02–22.68, P = 0.042).

Conclusion

There is a relationship between the metabolic characteristics of the tumor and its aggressiveness. According to our results, patients with oral cavity carcinomas with low transcriptional expression levels of PDHB have a significantly higher risk of local tumor recurrence.

Resumen

Antecedentes

La alteración del metabolismo celular es una de las características distintivas de las células cancerígenas, y favorece el proceso de la glucólisis aeróbica, conocido como efecto de Warburg. El complejo de piruvato deshidrogenasa (PDH) es uno de los elementos implicados en este proceso metabólico. El objetivo del presente estudio es evaluar la relación entre la expresión transcripcional de PDHB y el riesgo de recidiva local en los pacientes con cáncer en la cavidad oral.

Métodos

Determinamos la expresión transcripcional de PDHB en biopsias de 41 pacientes con cáncer en la cavidad oral tratados con cirugía. Se categorizó la expresión de PDHB de acuerdo con el control local de la enfermedad, con un análisis de partición recursiva.

Resultados

Durante el periodo de seguimiento, trece pacientes (31,7%) tuvieron una recidiva local del tumor. Considerando el control de la enfermedad local como variable dependiente, el análisis de partición recursiva clasificó a los pacientes en dos categorías, de acuerdo con la expresión de PDHB alta (n = 16, 39%) o baja (n = 25, 61%). La tasa de supervivencia libre de enfermedad a cinco años con expresión alta de PDHB fue del 84,8% (95% IC: 65,2–100%), siendo del 54,3% (95% IC: 34,3–74,2%) (P = 0,034) para los pacientes con expresión baja. Los resultados del análisis multivariante reflejaron que los pacientes con expresión baja de PDHB tuvieron un riesgo 4,90 veces mayor de recidiva local del tumor (95% IC: 1,02–22,68, P = 0,042).

Conclusión

Existe una relación entre las características metabólicas del tumor y su agresividad. Conforme a nuestros resultados, los pacientes con cáncer en la cavidad oral y bajos niveles transcripcionales de PDHB tienen un riesgo significativamente mayor de recidiva local del tumor.

Keywords

Pyruvate dehydrogenase-betaPDHBAerobic glycolysisWarburg effectOral cavity carcinomaLocal recurrence

Palabras clave

Piruvato deshidrogenasa-betaPDHBGlucólisis aeróbicaEfecto de WarburgCáncer en la cavidad oralRecidiva local

Introduction

The treatment of choice for patients with squamous cell carcinoma of the oral cavity is surgery, combined with adjuvant radiotherapy or chemo-radiotherapy depending on the pathological findings.1 One of the main causes of therapeutic failure in patients with oral cavity carcinoma is the local recurrence of the tumor. Among the prognostic factors that have been associated with local recurrence after surgery are the status of the resection margins2 and the depth of invasion,3 which are the main variables considered in determining the need for adjuvant treatment.

To dispose of a biomarker with the ability to predict an increased risk of local recurrence would help to determine the appropriateness and intensity of adjuvant treatment, as well as to define more strict follow-up protocols in high-risk patients.

Glucose is one of the major energy sources used by cells. The two main metabolic pathways that provide energy to the cell are glycolysis, under aerobic or anaerobic conditions, and the Krebs Cycle and subsequent oxidative phosphorylation under aerobic conditions. Glucose is metabolized to pyruvate by glycolysis and under aerobic conditions this is processed at the mitochondrial level by the Krebs Cycle, which counts as an initial step the enzymatic activity of the pyruvate dehydrogenase (PDH) complex. On the contrary, in anaerobic conditions the transformation of pyruvate into lactate is favored, a less efficient process from an energetic point of view.4

Otto Warburg observed that tumor cells had a high appetite for glucose, and a preference to metabolize it via glycolysis to lactate even in the presence of oxygen, a mechanism that has come to be known as the Warburg effect.5 This reprogramming of cellular metabolism towards aerobic glycolysis or Warburg effect is one of the modifications developed by tumor cells to survive in an unfavorable microenvironment while retaining their proliferative capacity.6

This metabolic shift of the tumor cell in favor of aerobic glycolysis is extensively regulated. One of the elements involved in this regulation is the activity of the PDH complex, which is composed of several enzymes, including the PDH-beta subunit, encoded by the PDHB gene. In turn, the activity of the PDH complex is regulated by the action of specific kinases (PDK), which cause inactivation by reversible phosphorylation, and phosphatases that restore the catalytic activity of the PDH complex.7

There is evidence from in vitro tumor models that suppression of the PDH complex is associated with an increase in the activity of the glycolysis pathway. As a consequence, there is an increase in lactate concentrations in the tumor microenvironment. This rise in lactate concentration induces an alteration in the phenotypic behavior of the tumor cells, with a decrease in intercellular adhesion, an increase in migration capacity, an increase in extracellular matrix degradation at the expense of metalloproteinases activation, a reduction in sensitivity to pro-apoptotic signals, and an increase in evasiveness to the immune response.8, 9, 10

We hypothesize that tumors with a reduction in PDH complex activity and a consequent increase in the glycolysis pathway would have an increased aggressiveness and local infiltration capacity that would favor tumor recurrence. The present study aims to evaluate whether there is a relationship between tumor aggressiveness manifested as the risk of local

recurrence and the metabolic status analyzed from the transcriptional expression of PDHB, one of the limiting enzymes in the mitochondrial phosphorylation pathway.

Material and methods

Patients

We carried out a retrospective study based on biopsy samples from 41 consecutive patients with squamous cell carcinoma located in the oral cavity and treated surgically in our center during the period 2005−2015. All patients were evaluated by the Oncology Committee of our center, which indicated the treatment according to institutional protocols. Table 1 shows the characteristics of the patients included in the study. Given the interaction in tobacco and alcohol consumption, a combined variable of toxics consumption was created with three categories: no consumption; moderate consumption (<20 cigarettes/day and/or <80 g alcohol/day); and severe consumption (≥20 cigarettes/day or ≥80 g alcohol/day). Margins with less than 5 mm of healthy tissue were considered as close, and those in which the tumor reached the resection limit were considered as positive. The criteria for adjuvant treatment were pT4 tumor extension, positive or close resection margins, and the presence of lymph node metastases with extracapsular spread. All patients included in the study had a simultaneous surgical treatment on the lymph node areas which consisted of a unilateral (n = 24) or bilateral (n = 17) neck dissection.

Table 1. Characteristics of the patients included in the study.

Empty Cell	Nº patients (%)
Age	Mean 69,5 years/range 31,1–97,7 years
Gender Men	26 (63.4%)
Female	15 (36.6%)
Sub-localizationFloor of mouth	13 (31.7%)
Tongue	15 (36.6%)
Retromolar trigon	7 (17.1%)
Buccal mucosa	6 (14.6%)
Toxic consumption No	10 (24.4%)
Moderate	16 (39.0%)
Severe	15 (36.6%)
Local extension cT1-2	11 (26.8%)
cT3	13 (31.7%)
cT4	17 (41.5%)

Regional extension cN0 16 (39.0%)

cN+ 25 (61.0%)

Resection margins Negative 32 (78.0%)

Near 6 (14.6%)

Positive3 (7.3%)

Histologic grade Well differenced 5 (12.2%)

Moderately differenced33 (80.5%)

Poorly differenced 3 (7.3%)

Adjuvant treatment No 18 (43.9%)

Radiotherapy 17 (41.5%)

Chemo-radiotherapy 6 (14.6%)

Data related to the characteristics of the patients was obtained retrospectively from a database that prospectively collects information regarding treatment and follow-up of patients with a malignant head and neck tumor treated at our center since 1985.11

Transcriptional analysis

The biopsy samples were obtained from the primary tumor site at the beginning of the surgical procedure, immediately included in RNA-later (Quagen GmbH, Hilden, Germany) to prevent mRNA degradation, and stored at −80°C until processing. Total RNA was extracted using Trizol (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The cDNA was obtained by reverse transcription of 1 µg of RNA with High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, USA), and transcriptional expression of PDHB and Beta-actin as an endogenous control was assessed by RT-PCR on an ABI Prism 7000 using pre-designed validated assays (TaqMan Gene Expression Assays; Applied Biosystems).

Statistical analysis

We compared the transcriptional expression values of PDHB according to toxics consumption, local and regional extension of the tumor, histologic grade, and local control of the disease. Comparisons were made using the Student's t-test and the ANOVA test.

The continuous value of PDHB transcriptional expression was assessed with a recursive partitioning analysis using a classification and regression tree model considering the local control as the dependent variable. Local recurrence-free survival was calculated according to the categories obtained with recursive partition analysis with the Kaplan-Meier method, using the log-rank test in the comparison of survival curves. We carried out a multivariate analysis considering local recurrence-free survival as the dependent variable, and the local and regional extension of the tumor, the status of resection margins, adjuvant treatment, and PDHB transcriptional expression category as independent variables.

Results

No significant differences appeared in PDHB transcriptional expression levels according to toxics consumption (P = 0.627), local (P = 0.806) or regional (P = 0.937) extension of the tumor, or histological grade (0.512). Table 1 of the supplementary material shows the PDHB expression values as a function of the different variables.

During the follow-up period, 13 patients (31.7%) had a local recurrence of the tumor. Patients with local recurrence tended to low PDHB transcriptional expression levels, but the differences did not reach statistical significance (P = 0.190). Fig. 1 in the supplementary material shows the distribution in PDHB expression values depending on local disease control.

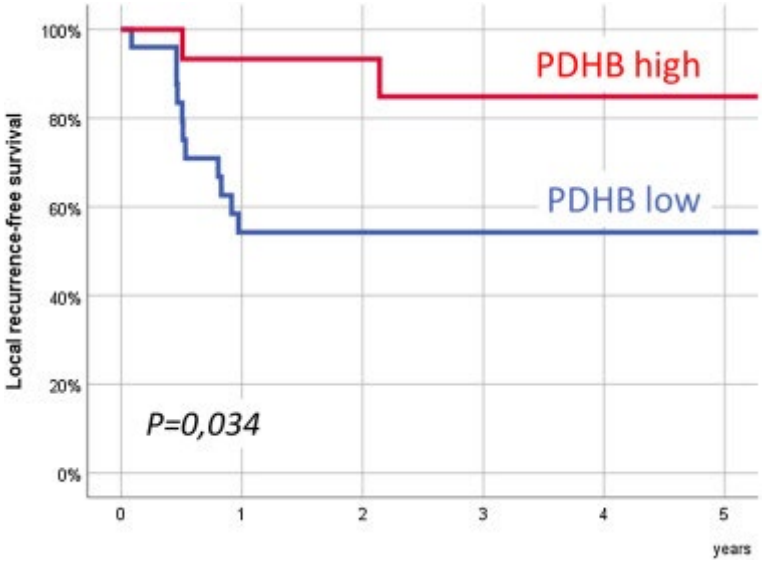

Figure 1. Local recurrence-free survival according to the PDHB expression category.

Considering local disease control as the dependent variable, the recursive partitioning analysis classified the patients in two categories according to high (n = 16, 39.0%) or low (n = 25, 61.0%) PDHB transcriptional expression levels. Fig. 1 shows the local recurrence-free survival as a function of the PDHB expression category. Five-year local recurrence-free survival for patients with high PDHB expression was 84.8% (95% CI: 65.2–100 %), significantly higher than for patients with low expression, which was 54.3% (95% CI: 34.3–74.2 %) (P = 0.034).

These differences in local disease control were not reflected in specific survival. The 5-year specific survival for patients with high PDHB expression was 59.7% (95% CI: 34.3–85.1 %) and for patients with low expression was 47.4% (95% CI: 27.7–67.1 %) (P = 0.253). No differences in regional (P = 0.823) or distant (P = 0.549) recurrence-free survival appeared as a function of the PDHB expression category.

Table 2 shows the results of the multivariate analysis. The only variable that was significantly related to the local control of the disease was the PDHB transcriptional expression category. Compared to patients with high PDHB expression, patients with low expression levels had a 4.90-fold increased risk of local recurrence of the tumor (95% CI: 1.02–22.68, P = 0.042). The other variables that showed a tendency to be related to local recurrence were positive or close resection margins (P = 0.124) and cT4 tumor extension (P = 0.061), but without the HR value reaching statistical significance.

Table 2. Results of the multivariate study considering the local recurrence-free survival as the dependent variable.

Empty Cell		HR	CI 95% HR	P
Local extension	cT1-2	1	Empty Cell	
cT3	1.14	0.14–8.99	0.900	
cT4	5.94	0.92–38.27	0.061	
Regional extension		N0	1	Empty Cell
N+	1.31	0.35–4.85	0.686	
Resection margins		Negative	1	Empty Cell
Near or positive		2.55	0.77–8.42	0.124
Adjuvant treatment		No	1	Empty Cell
Yes	0.81	0.15–4.44	0.817	
PDHB	High	1	Empty Cell	
Low	4.90	1.05–22.68	0.042	

HR, hazard ratio.

Discussion

According to our results, low levels of PDHB transcriptional expression in oral cavity carcinomas were associated with more aggressive behavior, resulting in an increased risk of local tumor recurrence.

It has been described that reduced PDHB expression is associated with increased cell proliferation, migration, and invasion capacity in tumor models of colorectal carcinoma,12 melanoma,13 or glioma.14 In addition, increased transcriptional expression of PDHB has been associated with increased survival in patients with clear cell renal carcinoma.15

Experimental in vitro studies performed with head and neck carcinoma cell lines have shown that reduction in PDH complex activity induced the acquisition of a Warburg phenotype with a metabolic predominance of glycolysis, an increase in cell proliferation, migration, and invasion

capacity.16, 17, 18 In vivo, injection of tumor cells with decreased expression of PDHB (beta subunit)17 or PDHA (alpha subunit)18 led to the formation of more voluminous tumors, translating an increase in cell proliferation. Moreover, in an experimental model of head and neck carcinoma, overexpression of PDH complex led to a dramatic reduction in tumor growth.16 In oropharyngeal carcinoma cell lines differences have been shown in the metabolic pattern as a function of HPV status. HPV-positive lines tended to metabolize glucose by the mitochondrial respiration pathway, whereas HPV-negative lines were dominated by the glycolysis pathway.19 HPV-negative lines had a significant increase in the expression of PDH kinases (PDHK), specific inhibitors of PDH complex activity.

In addition, in vitro studies with head and neck carcinoma lines have shown a relationship between the cellular metabolic profile and resistance to treatments. Cell lines resistant to cisplatin,18, 20 taxol,18 or cetuximab21 had significantly lower levels of PDH complex activity than parental lines, with reversal of this drug resistance after PDH expression restoration. Furthermore, in an HPV-negative oropharyngeal carcinoma line, suppression of the inhibitory activity of the PDH complex exerted by specific kinases led to a significant increase in sensitivity to radiotherapy.19

Besides the experimental findings, the clinical results obtained in patients with head and neck carcinomas support a more aggressive behavior associated with a reduction in PDH activity. In a study of a cohort of 140 patients with head and neck squamous cell carcinomas, Wigfield et al.22 determined the immunohistochemical expression of PDH kinase 1 (PDHK1), a specific negative regulator of PDH activity. According to their results, patients with elevated PDHK1 expression had a significantly higher risk of recurrence (51.8% versus 30.5%, P = 0.01) and significantly reduced specific survival (P = 0.005). In a similar study in 212 patients with surgically treated oropharyngeal carcinomas, Golias et al.23 reached similar conclusions, with a significant reduction in overall survival in the group of patients with elevated PDHK1 expression (P = 0.0496). Finally, in 102 patients with rhinopharyngeal carcinoma, Xiang et al.24 found a significant reduction in the specific survival in the group of patients with elevated immunohistochemical expression of PDHK1 (P = 0.015). Although none of the studies directly evaluate the expression of the PDH complex, they all agree in pointing out a significant relationship between elevated expression of a specific inhibitor of the pathway such as PDHK1 and a significant decrease in survival.

Our study is the first to analyze the relationship between PDHB transcriptional expression and prognosis in patients with surgically treated oral cavity carcinomas. According to our results, a low expression of PDHB is associated with an increase in local aggressiveness, which translates into a significant increase in the risk of local recurrence of the tumor. In contrast, we found no relationship between PDHB expression and regional or distant disease control. Based on the result of the multivariate study, the PDHB expression category was the only variable that was significantly related to the risk of local recurrence, with patients with low expression having an almost 5-fold increased risk of recurrence (95% CI: 1.05–22.68, P = 0.042). The other variables that were associated with an increased risk of local recurrence were close or positive resection margins and an advanced local extension category of the tumor (cT4). Table 2 of the supplementary material shows the result of a multivariate analysis in which the pathological loco-regional extension categories (pTNM) were included as variables. The results obtained were similar, although in this analysis the Hazard ratio of the PDHB expression category did not reach statistical significance (P = 0.094).

Our results and those obtained by the other authors support the existence of a relationship between the metabolic characteristics of the tumor and the prognosis of the disease in patients with carcinomas of the oral cavity. Tumors with a metabolic profile that favors glycolysis at the expense of oxidative phosphorylation at the mitochondrial level (Warburg effect) would have lower local control of the disease.

One element to highlight is the possibility of therapeutic action on this metabolic pathway, with drugs with PDH kinase inhibitory capacity that would ultimately aim to restore PDH activity.25

The appearance of a local recurrence in patients with negative resection limits could be due to two situations: (1) the presence of microscopic clusters of invasive cells disconnected from the clinical tumor infiltration front which remain beyond the surgical resection margin (skip local metastasis); (2) the dedifferentiation of the mucosa adjacent to the resection area, which although from a clinical and histological point of view would not present evident alterations at the time of surgery, it would contain lesions at the molecular level that promote the reappearance of the tumor (cancerification field).

The increase in migration and invasion capacity of the tumoral cells could be one of the causes that justify the increased risk of local recurrence in the group of patients with reduced PDHB expression levels, being consequently these recurrences related to the concept of local skip metastasis. It could be speculated that cases of recurrence related to the local persistence of tumor deposits (local skip metastasis) would manifest clinically more rapidly than those secondary to dedifferentiation of the marginal mucosa (cancerification field). To test this hypothesis, we compared the local disease-free periods for patients with local recurrence depending on the level of PDHB expression. We found that the mean local disease-free survival for those patients with low PDHB expression ($n = 11$) was 0.59 years, compared to a mean period of 1.3 years for patients with high PDHB expression ($n = 2$), with the differences reaching statistical significance ($P = 0.048$). With all the precautions that the limited number of patients analyzed entails, these results seem compatible with the hypothesis that recurrences in patients with reduced PDHB expression would be the consequence of microscopic local persistence of the tumor beyond the limits of the surgical resection.

Our study has several limitations that should be considered when interpreting its results. It is a retrospective study conducted in a limited cohort of patients, so it should be considered as a preliminary study that requires an increase in the number of patients analyzed and external validation. On the other hand, we have only carried out a determination of the transcriptional expression of the PDHB, and there may be post-transcriptional regulatory mechanisms with the capacity to modify the results.

Nevertheless, this is an evaluation of a homogeneous cohort of patients treated consecutively in a single center and with follow-up data obtained prospectively, which ensures the quality of the information. Our results point to the possibility of developing biomarkers based on the metabolic characteristics of the tumor with predictive capacity for response to surgery in patients with oral cavity carcinomas. If validated, this predictive capacity would serve to modulate the appropriateness and intensity of adjuvant treatments, as well as to adapt follow-up protocols to the patients' risk of recurrence.

Conclusion

There is a relationship between the metabolic characteristics of the tumor and its aggressiveness. According to our results, patients with oral cavity carcinomas with low transcriptional expression levels of PDHB have a significantly higher risk of local tumor recurrence.

Conflict of interest

The authors have no conflicts of interest.

Ethics approval and consent to participate

The study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. The study was approved by the Hospital de Sant Pau Ethics Committee for Scientific Research (IIBSP-CCC-14-93). Informed consent was obtained from all individual participants included in the study.

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