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Original article

TKTL-1 expression in lung cancer

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ABSTRACT

Study of the physiological changes associated with the development of malignancy demonstrates a metabolic signature for the different stages of tumorigenesis. Increased glucose uptake and lactate production have been detected during malignant transformation. Based on energy production, malignancies can be divided into two subclasses: (a) tumor cells which use the mitochondrial machinery for ATP synthesis, and (b) tumor cells which generate ATP by glucose fermentation and lactate production even in the presence of oxygen (aerobic glycolysis). Recently, transketolase-like protein 1 (TKTL1) gene expression has been shown to contribute to carcinogenesis through increased aerobic glycolysis and hypoxia-inducible factor alpha stabilization. In the present study, 197 patients suffering from lung cancer were investigated by immunohistochemistry for the presence of TKTL1 protein expression. We detected: (1) moderate to strong TKTL1 expression (immune reactive score > 100) in 39.1% of the investigated lung cancer patients; (2) a complete lack of TKTL1 by immunohistochemistry in 12.7% of lung cancer cases, with small cell lung cancer (SCLC) being most frequent in this subgroup; (3) no correlation of TKTL1 with overall survival, disease-free survival, any of the established variables of the TNM system, grading, stage, smoking status, or gender. Based on this data, we conclude that TKTL1 could be a target protein for improved therapeutic strategies in some cases of lung cancer.
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Introduction

Lung carcinoma represents one of the leading causes of cancer-related deaths in both men and women [19,31] with disappointing survival data. The five-year survival for all lung cancer stages, irrespective of surgical intervention, is below <10% in the *Onkologischer Schwerpunkt Stuttgart* (OSP) retrospective data collection [22,26]. Even when evaluating only patients undergoing surgical resection, the five-year overall survival is only 40% (95% CI interval 36–44) as shown by Birim et al. [2]. The standard treatment for lung cancer consists of thoracic surgery, chemotherapy, and radiation; this approach could be improved by new treatment concepts, such as targeted therapy with tyrosine kinase inhibitors [3,4,15,16,17,28].

Investigations of protein expression in lung cancer with the potential of targeted cancer treatment are of intense clinical interest. The expression of possible target proteins in lung cancer should be considered with regard to two aspects:

(1) Proteins in tumor cells may give information about the prognosis of a given patient beyond TNM classification.
(2) The expression of a protein may be a predictor for treatment decisions, similar to the demonstration of steroid receptors or c-erbB2 expression in tumor cell membranes [1,29] in breast cancer patients as prerequisite for a anti-hormonal therapy or antibody treatment.

Originally described by Otto Warburg more than 80 years ago, the importance of tumor bioenergetics has recently experienced a revival in cancer research [12–14]. Common genetic aberrations in tumors have been demonstrated to contribute to a cancer-specific metabolism, which is characterized by high glucose consumption and lactate production in the presence of oxygen (aerobic glycolysis or Warburg effect) [39]. In cancer diagnostics, aerobic glycolysis is already being exploited for the detection of tumors and metastases using fluoro-deoxyglucose (FDG) and positron

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emission tomography (PET) (for an overview, see Kelloff et al. [21]). In summary, the Warburg effect is well recognized as a hallmark of advanced cancer, but so far this knowledge has not lead to the development of targeted therapies, at least for lung cancer patients.

Aerobic glycolysis has also been detected in healthy tissues, such as the testis and retina. Transketolase-like-1 (TKTL1) protein is expressed in both healthy tissues executing aerobic glycolysis, as well as in advanced cancers with aerobic glycolysis. TKTL1 is the result of a TKT gene duplication followed by deletion of a former coding exon present in all transketolase proteins. Using a knock-out mouse model, TKTL1 was shown to protect healthy cells from damage [3]. TKTL1 gene expression in cancer cells contributes to carcinogenesis through increased aerobic glycolysis and HIF1alpha stabilization [31].

TKTL1 is expressed in different types of solid carcinomas, such as those of the uterine cervix [7], renal cell [23], urothelium [23], stomach [36], colon [23], head and neck [38] and lung [32], and has been found to represent a prognostic marker for poor survival in colon and urothelial cancer [23]. Overexpression of TKTL1 defines a subgroup of tumors that are amenable to inhibition of aerobic glycolysis by using a substrate limitation (ketogenic diet) and small molecule-based inhibition (thiamin analogs such as oxythiamine, tocotrienols, and polyphenols) [6,8,9,12,34]. Since the basis of several mechanisms of tumor resistance to radiotherapy and chemotherapy is aberrant glucose metabolism, the reactivation of a more 'normal' oxidative (mitochondrial) metabolism could revert tumors to chemo- and radiosensitivity. TKTL1 protein expression has also been exploited for identification of cancer patients with an increased glucose metabolism in primary tumors and metastases. Therefore, a possible therapeutic approach justifies further investigations of TKTL1 expression in lung cancer patients [40].

In this work we demonstrate the presence of TKTL1 protein in lung cancer cells, indicating cancer patients eligible for a targeted cancer therapy. We examined 197 different lung cancer samples for specific TKTL1 protein expression by immunohistochemistry. We found that a marked subset of patients had TKTL1 over-expressing tumors, and that TKTL1 over-expression did not correlate with variables affecting disease outcome, such as DFS (disease free survival) or OVS (overall survival).

Material and methods

Patients

One hundred ninety-seven patients diagnosed with lung cancer from 1994 to 1996 were randomly chosen from the Department of Thoracic Surgery for inclusion. In 148 patients, material from either segmental resection, lobectomies, bilobectomies, or pneumectomies was investigated. In 45 cases of small cell carcinoma or stage IV non-small cell carcinoma, only biopsies of the tumor tissue were evaluated. Table 1 reports patients' clinical data. Mean age of tumor patients was 61.2 ± 9.0 years. Follow-up ranged from 0 to 74.4 months overall. Follow-up for survivors was 33.3 ± 22.4 months (median = 36.9); follow-up for non-survivors was 14.2 ± 12.8, 11.2 months (median = 10.7). Mean OVS was 24.0 months ($\pm$20.8), and median OVS was 20.8 months. Mean DFS was 20.8 months ($\pm$20.7), and median DFS was 11.1 months. Overall, 47.8% of patients died ($N = 94$). The cause of death was not classified as tumor-related or not tumor-related in patient records.

Staining methods

Immunohistochemical staining for TKTL1 was performed by a two-step peroxidase-labeled dextran-polymer method (Envision, Dako Denmark). Tumor sections (3 μm) of patients suffering from lung cancer were pre-treated for antigen retrieval at pH 6 (PBS buffer) for 30 min. A mouse monoclonal antibody anti-TKTL1 (clone JFC12T10) [10,11] was diluted 1:30. Sections were incubated with the antibody for 30 min. After rinsing in PBS buffer, the sections were incubated with a peroxidase-labeled dextran polymer. Peroxidase was demonstrated by incubation in H_2O_2/diaminobenzidine, resulting in a brown final reaction product. Nuclei were counterstained with hematoxylin. Testis tissue was used as a positive control. Omitting the first antibody, as well as the secondary dextran polymer or both, resulted in constant negative staining patterns. Method specificity was controlled by cytokeratin 18 (Dako, Hamburg, Germany).

Assessment of TKTL1

Tumor cell staining was rated according to the frequency of positive tumor cells (0–100% in 10% steps) and staining intensity ranging from 0 to 3 (0: no staining to 3: strong staining). An immunohistochemical score index was calculated by multiplication of both parameters to characterize TKTL1 expression (immunoreactive score, IRS). The IRS ranged from 0 to 300. For normal tissue, the expression of TKTL1 was classified as either absent, weak, or moderate/strong. The following normal lung structures were evaluated: bronchial epithelium, submucosa, vessel and smooth muscle, sero-mucous glands, and pneumocytes. TKTL1 expression was considered weak when IRS scores were 1–90. Moderate TKTL1 expression was defined as a score of 100–190. Values ranging from 200 to 300 were considered strong expression. In a second approach, we compared tumors expressing IRS scores below 150 with those $\geq$150.

Statistical analysis

All data were analyzed in S+. We applied two-side *t*-test, chi^2 test, or Fisher's test for canonical data, and Kaplan–Meier and Cox regression analysis for survival analysis. p values < 0.05 were considered statistically significant, and $p < 0.001$ was considered highly significant. Comparison between two independent observers was calculated by linear regression. OVS was measured as the time between surgery or diagnosis and death or censoring. DFS was defined as the time between either surgery or diagnosis and death, local, or distant metastasis. Testing for co-linearity was done with a chi^2 test. Survival data of OPS were analyzed by OCDM software [22].

Results

Patients

As demonstrated in Table 1, stage, TNM status, nodal status, and residual status predicted disease outcome on univariate analysis. Most patients were male (151; 76.6%) and smokers (159; 80.7%). One-, three-, and five-year survival rates for all stages were 70.8% (95% CI: 64.4–77.8), 47.6% (CI: 40.4–55.9), and 40.2 (CI: 32.13–50.4), respectively. In advanced cases (stage IV), one- and three-year survival rates were 43.35 (95% CI: 25.6–73.0) and 19.8 (95% CI: 6.6–59.1). Five-year survival could not be calculated for advanced stage patients due to the low number of patients at risk. The median of survival was not reached for stage I tumors and was 53.0 months for stage II, 12.7 months for stage III, and 5.3 months for stage IV. Most tumors were of squamous subtype. Most NSCLC were histological grade two. Small cell carcinomas are poorly differentiated by definition (see Table 1). Overall, 12.3% of patients (compared to 51% of the normal healthy population) [37] were female, and 23.4% were non-smokers (never smoked; compared to 35% in the normal

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