Expression of Ceramide Galactosyltransferase (UGT8) in Primary and Metastatic Lung Tissues of Non-Small-Cell Lung Cancer

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Abstract

Ceramide galactosyltransferase (UGT8) is an enzyme that regulates the synthesis of sphingolipids of the myelin sheath in nervous systems. The protein raises an increasing research interest as a potential marker of cancer progression in various organs. In the present study we seek to determine whether UGT8 could play a role of a therapeutic marker in non-small cell lung carcinoma (NSCLC). We addressed the issue by examining the intensity of UGT8 expression in tissue specimens of primary and corresponding metastatic lung tumors in 19 NSCLC patients undergoing surgery. The methodology was one of immunohistochemical tissue staining using light microscopy. The findings were that the majority of both lung primary and metastatic tumor tissues were positive in UGT8 signals. The cytoplasmic expression of UGT8 was found in 68.4 % of cases of primary tumors and 82.2 % of metastases, with a positive correlation between the UGT8 expression in both tumor tissues. The normal tissue adjacent to tumors showed no positive UGT8 staining. However, we failed to find any appreciable difference in UGT8 expression depending on the clinical stage of NSCLC or

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lymph node involvement. Nor was there any association between UGT8 expression in tumor tissues and patients' survival time. We conclude that it is unlikely that therapeutic targeting of UGT8 could inhibit cell proliferation and invasion of NSCLC. UGT8, although enhanced in NSCLC tissues, does not meet the criteria of a lung tumor marker. Thus, UGT8 cannot be considered as having diagnostic or therapeutic utility in NSCLC. The pathophysiological meaning of enhanced expression of UGT8 in lung cancer remains to be explored in further studies.

1 Introduction

Lung cancer is the most common malignancy and is the first cause of cancer deaths among men and the second among women (Jemal et al. 2011). The prognosis is poor and it mainly depends on the histologic type and clinical stage of disease (Kosacka and Jankowska 2007). The basic diagnostic method used today is immunohistochemistry enabling to determine the expression of tumor markers. In case of lung cancer, determination of expression of specific proteins also gives clues on the possible use of targeted immunotherapy, enables the monitoring of disease progression, and allows predicting the effects of therapy (Jassem et al. 2010). The ceramide galactosyltransferase protein (UGT8) has been originally reported as a myelin basic protein in the oligodendrocyte lineage in the central nervous system and in Schwann cells of the peripheral nervous system (Bosio et al. 1996; Dyer and Benjamins 1989). The protein is responsible for the synthesis of the glycosphingolipid-galactosyloceramid (GalCer). In the physiological condition, GalCer takes part in muscle cell differentiation by regulating calcium levels, and it affects the rebuilding of the cellular cytoskeleton (Schulte and Stoffel 1993). The elevated level of GalCer is observed in certain pathological conditions, notably in many types of cancer lesions. Initially, increased expression of GalCer has been reported in astrocytomas and oligodendrogliomas. Then, GalCer has also been demonstrated in mammary gland cancers and in metastatic lesions of prostate cancer (Dzięgiel et al. 2010; Ruckhaberle et al. 2009; Oudes et al. 2005; Sung et al. 1995). The expression of GalCer synthase is increased about 11-fold in ovarian epithelial carcinoma cells compared with normal ovarian stromal tissue (Liu et al. 2010).

The level of UGT8 expression positively correlates with increased risk of metastasis of primary mammary gland to the lungs (Dzięgiel et al. 2010). Nonetheless, UGT8 expression in primary and metastatic tumors of non-small cell lung cancer (NSCLC) has not yet been studied. In the absence of a satisfactory treatment outcome of patients with NSCLC, we deemed it warranted to investigate the possibility that UGT8 expression could play a potential role of a therapeutic marker in NSCLC. In the present study, we addressed the issue by examining the intensity of UGT8 expression in the specimens of primary and metastatic tumor tissues in NSCLC patients undergoing surgery, and the possible association of UGT8 with the clinico-pathological features.

2 Methods

The study was approved by the Bioethics Committee of Wroclaw Medical University in Wroclaw, Poland, and was conducted in accord with the principles set by the declaration of Helsinki for Human Research. This was a retrospective study that covered 19 patients (6 women

and 13 men) of the mean age of 64 ± 9, range 44–78 years. There were 5 patients below 60 and 14 above 60 years of age. All patients were treated in the Lower Silesian Center of Lung Diseases in Poland in the years 2001–2013. Lung tissue specimens were collected during surgical lobectomies of primary NSCLC and then during reoperations due to metastatic pulmonary lesions, performed after 1–93 months after the first tumor removing surgery. Clinico-pathological data of patients, obtained from the hospital archives, are depicted in Table 1.

Specimens were investigated by immunohistochemistry. Reactions were carried out in 4 µm thick paraffin tissue sections. For deparaffinization, rehydration, and exposure of antigenic determinants, glass slides were boiled in an alkaline buffer (pH = 9), a target retrieval solution, for 20 min at 97 °C, using a Pre-Treatment Link Station (DakoCytomation; Glostrup, Denmark). Then, after using the Flex EnVision Peroxidase-Blocking Reagent for 5 min (DakoCytomation; Glostrup, Denmark), immunohistochemical reactions were carried out with primary polyclonal rabbit anti-UGT8 antibodies (20 min, room temperature, dilution 1: 700; Prestige Atlas Antibodies; Stockholm, Sweden) and secondary horseradish peroxidase-conjugated antibodies (20 min; EnVision FLEX/HRP, DakoCytomation). The slides were developed with diaminobenzidine (10 min). Contrasting staining was performed using EnVision FLEX hematoxylin (5 min). Immunohistochemical reactions were examined under light microscopy (Olympus BX-41; Tokyo, Japan). The cytoplasmic expression of the UGT8 protein was evaluated using a semi-quantitative scale designed by Remmele and Stegner (1987); details of which are set out in Table 2.

An analysis of data distribution was performed with the Shapiro-Wilk test and differences between groups were tested with the Mann-Whitney U test. Spearman's correlation coefficient was used to assess the relationship between the UGT8 expressions in primary tumor *vs*. metastases. The Kaplan-Meier survival curves were used to define the probability of patient surviving depending on the low or high level of UGT8 expression. The differences in results were considered statistically significant at $p < 0.05$. Statistical analysis was performed using the commercial statistical package Graph Pad Prism ver. 5.0.

3 Results

We examined tissues of 19 primary tumors and 19 corresponding metastatic lung cancer foci. Cytoplasmic expression of UGT8, exemplified in Fig. 1, was found in 68.4 % of cases of primary tumors and 82.2 % of metastases. Thus, the majority of lung tumor tissues were positive in UGT8 signals as opposed to adjacent normal tissues. The normal tissue showed virtually no positive UGT8 staining, irrespective of whether the tumor staining was distinctly strong or mild as demonstrated in Fig. 1. Scoring of the staining intensity corresponded to moderate or strongly positive immunoreaction. The mean UGT8 expression in primary tumors amounted to 2.7 ± 3.3, with the median of 2.0 (95 % CI 1.1–4.4). For comparison, the mean score of UGT8 expression in metastatic tumors was 3.5 ± 3.3, and the median was 3.0 (95 % CI 1.9–5.1) (Fig. 2). Increasing expression of the UGT8 protein in primary tumors was associated with its being increased also in the corresponding

Table 1 Clinico-pathological features of non-small cell lung cancer

Histopathological type	n
Squamous cell carcinoma	6
Adenocarcinoma	12
Macrocellular carcinoma	1
TNM	
T1	5
T2	6
T3	1
Tx	7
N0	9
N1	2
N2	1
Nx	7
M0	4
Mx	15

Table 2 Semi-quantitative scale based on the intensity immunostaining score (IRS) designed by Remmele and Stegner (1987)

| Intensity of staining	Percentage of positive cells	IRS score (0–12)	IRS classification
0 = no color reaction	no positive cells	0–1 = negative	0 = negative
1 = mild reaction	<10 % positive cells	2–3 = mild	1 = positive, weak expression
2 = moderate reaction	10–50 % positive cells	4–8 = moderate	2 = positive, mild expression
3 = intense reaction	51–80 % positive cells	9–12 = strongly positive	3 = positive, strong expression
	> 80 % positive cells		
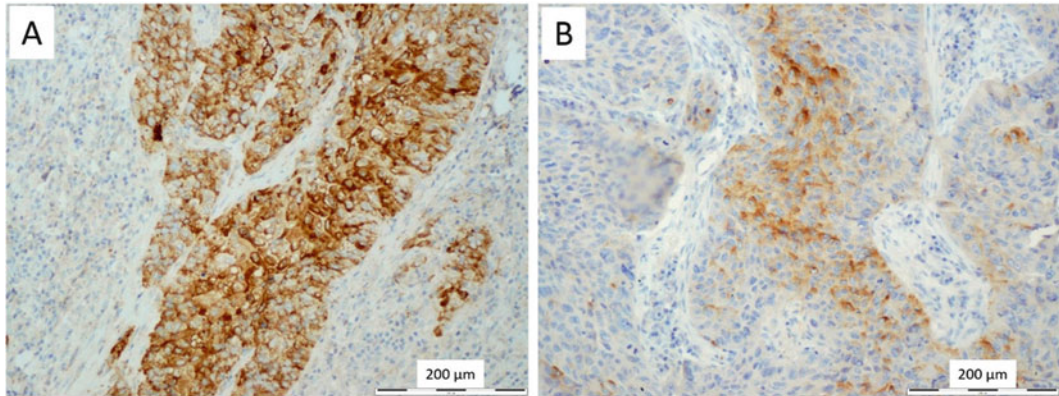
Fig. 1 Non-small cell lung cancer: (**a**) the primary site, (**b**) lung metastasis. Examples of strong and mild cytoplasmic UGT8 expressions; magnification x200

Fig. 2 UGT8 expression in primary and secondary tumors of non-small cell lung cancer (NSCLC). No difference between the two kinds of tissue was evident
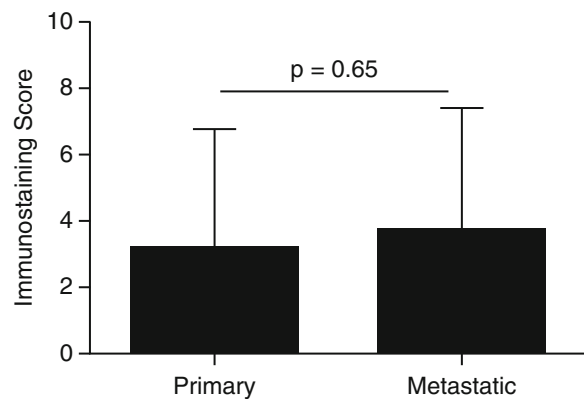
lung metastases, although the correlation was rather weak ($p < 0.05$; $r = 0.48$) (Fig. 3).

We failed to find any appreciable difference in the expression of UGT8 between primary tumors and metastases in the subgroups of patients with and without the involvement of lymph nodes, i.e., in clinical stage below and above IIB, in both male and female patients. There was no significant association between the patients' age and expression of UGT8 concerning both primary and metastatic tumors. Moreover, survival time of patients had no relation to the level of UGT8 expression. It remained similar in the patients with low and high expression of UGT8 in both primary and metastatic tumors (Figs. 4 and 5).

Fig. 3 Association of cytoplasmic UGT8 expression in non-small cell lung cancer (NSCLC); primary *vs*. metastatic lung tumor tissues
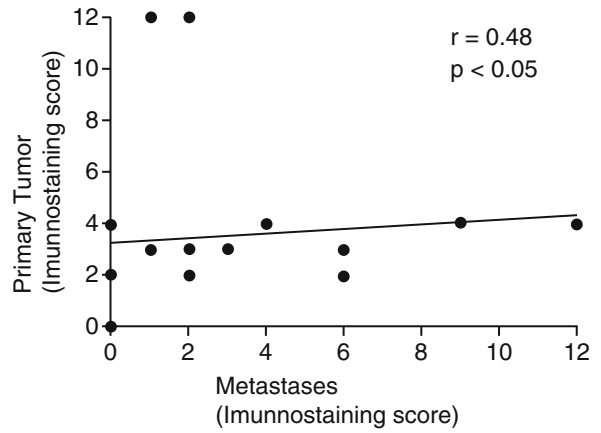
Fig. 4 The Kaplan-Meier survival curves of patients with primary tumor of non-small cell lung cancer (NSCLC) having low ≤ 3 and high > 3 immunostaining score of UGT8 expression. No significant difference between the two subgroups of patients was found
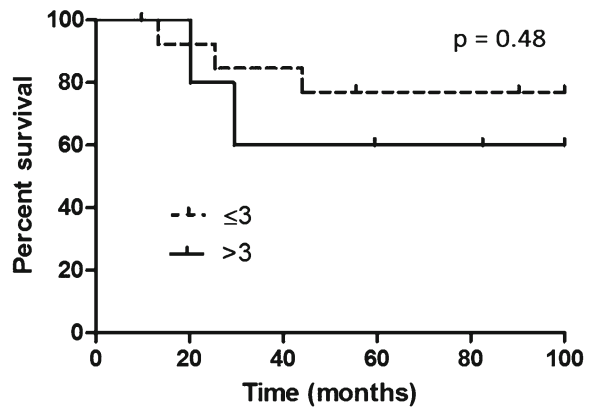
Fig. 5 The Kaplan-Meier survival curves of patients with lung metastases accompanying primary non-small cell lung cancer (NSCLC) having low ≤ 2 and high > 2 immunostaining score of UGT8 cytoplasmic expression. No significant difference between the two subgroups of patients was found
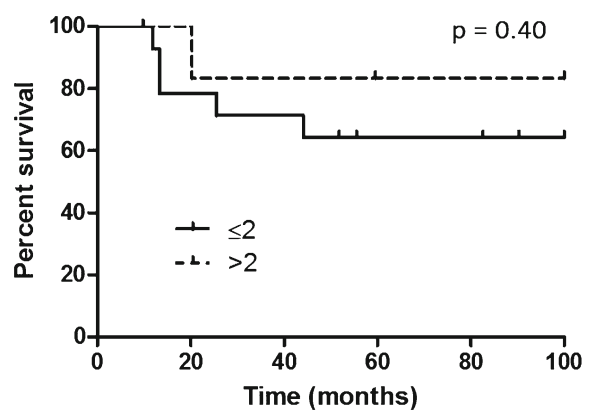
4   Discussion

In the present study we investigated the hypothesis that the cellular ceramide galactosyltransferase, UGT8, known to be engaged in tumorigenesis in various tissues, could potentially be a marker of progression in non-small cell lung cancer (NSCLC). To this end, we examined the immunohistochemical intensity of UGT8 expression in NSCLC and its metastases to the lungs, and the possible association of UGT8 with the clinical features of cancer. The study demonstrates the distinct presence of UGT8 of moderate intensity on the semi-quantitative scale of immunostaining of Remmele and Stegner (1987) in both primary NSCLC and its lung metastases. We also found that a high level of UGT8 in primary tumor was matched by a high level of UGT8 in metastases. We thus may say that overall there was a distinct increase in UGT8 expression in lung cancer tissues relative to adjacent normal lung tissue where the UGT8 expression was negligible or null. These results are, generally, in line with the results of other authors who have shown that the expression of UGT8, at both transcriptional and protein levels, is positively associated with the metastatic potential of pancreatic ductal adenocarcinoma cell lines but adjacent normal duct cells show no UGT8 expression (Li et al. 2013).

Other than the presence of UGT8 expression in cancerous lung tissue, the findings of the present study were largely negative and disappointing in terms of the possible therapeutic or marker-like role in NSCLC of UGT8. The expression of UGT8 did not appreciably differ between the primary and metastatic tumors, it had no relation to the cancer stage, assessed by the presence or lack of lymph node involvement, nor was it affected by patients' age or gender. Further, survival time of patients had no apparent relation to the magnitude of UGT8 expression. Therefore, it seems unlikely that therapeutic targeting of UGT8 could inhibit cell proliferation and invasion of NSCLC.

Ruckhaberle et al. (2009) have been the first who directed attention to the possible role of kinases involved in phospholipid activity and cancer progress. These authors have identified a group of enzymes such as sphingosine kinase-1 (SPHK1), ganglioside GD3 synthase, and the ceramide galactosyltransferase (UGT8) in the microarray studies of various subtypes of breast cancer cells. They have found that UGT8 is associated with a higher proliferation and fewer apoptotic cells in estrogen-negative breast cancer type, which is associated with a worse prognosis. Dzięgiel et al. (2010) have shown that higher expression of the UGT8 in breast cancer is associated with increased risk of metastases to the lungs. Other observations on the role of UGT8 in tumorigenesis are in line with the above outlined research. A higher degree of malignancy has been noted in breast cancer associated with a higher expression of UGT8 in dogs (Nowak et al. 2013). Likewise, Owczarek et al. (2013) in the experimental murine model of metastatic cancer cells of the breast (MDA-MB-231), with the induction of apoptosis with doxorubicin, have confirmed the association of UGT8 activity with a higher rate of cancer cell proliferation and fewer apoptotic cells; the conditions that promote survival of tumor cells and are conducive to metastases in distant organs. The polymorphism of the UGT8 gene apparently does not affect the effectiveness of chemotherapy in lung cancer, but it may increase the neutropenia of chemotherapy (Nakamura et al. 2011). In connection to those reports, Zheng et al. (2002) have reported the activity of the glucuronyl enzymes (UGT1A6) in detoxification of airway and lung tissues from carcinogenic metabolites of benzopyrene, present in tobacco smoke. The UGT1A6 gene polymorphism, expressed in leukocytes of patients with lung cancer, is associated with a higher probability of malignancy. Kua et al. (2012) have suggested that the

investigation of the polymorphisms of UGT1A6 gene may be used to detect people with increased risk for lung cancer. Other studies demonstrate that the frequency of the low activity alleles UGTA7*2 and UGTA7*3 of the UGTA7 gene is significantly higher in Taiwanese lung cancer patients than in healthy subjects. The reduced enzyme activity may hamper detoxification of carcinogens and by doing so may foster cancer progression (Lee et al. 2011).

Given the largely negative results of the present study concerning the potential role of UGT8 expression as a prognostic of NSCLC cancer development and survival we backed away from further dwelling on the issue by studying the expression of the related enzyme galactosyl-ceramide (GalCer), whose synthesis is dependent on the action of UGT8. GalCer has been reported to have proliferative and metastatic potential due to its antiapoptotic activity as well as the ability to induce chemotherapeutic resistance in breast cancer cell lines (Nowak et al. 2013; Owczarek et al. 2013; Dzięgiel et al. 2010; Ruckhaberle et al. 2009). The role of GalCer in lung tumorigenesis remains at present unknown.

The limitation of the present study was that the immunochemistry we employed was restricted to the use of a single antibody against UGT8 and we investigated the metabolism of only one enzyme involved in glycolipid metabolism. Nonetheless, we conclude that UGT8, although enhanced in NSCLC tissues, does not meet the criteria posed for tumor markers, and therefore its potential diagnostic or therapeutic use in NSCLC cannot be considered. The pathophysiological meaning of enhanced expression of UGT8 in lung cancer remains conjectural and it should be explored in another study designs.

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