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Increased expression of 17-beta-hydroxysteroid dehydrogenase type 1 in non-small cell lung cancer

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ABSTRACT

Objectives: Recent studies indicated that estrogens may influence the development of non-small cell lung cancer (NSCLC). The 17-beta-hydroxysteroid dehydrogenase type 1 (HSD17B1) catalyzes the reduction of estrone (E1) to the highly potent E2. Although the significance of aromatase in an intratumoral E2 production in NSCLC is well established, the role of HSD17B1 remains largely unknown. Therefore, we investigated the expression of *HSD17B1* in lung cancerous and corresponding histopathologically unchanged tissues from NSCLC patients and the association between *HSD17B1* expression and clinicopathological features. Than, we examined the biological significance of HSD17B1 in NSCLC cells in vitro. We tested the impact of 5-Aza-2′-deoxycytidine (5-dAzaC) on HSD17B1 expression and activity.
Materials and methods: We used Real Time quantitative PCR (RT-qPCR), Western blotting and immunohistochemistry to evaluate *HSD17B1* expression in tissues obtained from 48 patients with NSCLC. The methylation status of the promoter region of *HSD17B1* in A549 and Calu-1 cells was evaluated by bisulfite sequencing. We investigated the effect of 5-dAzaC on HSD17B1 transcript levels (by RT-qPCR) and on HSD17B1 enzyme activity by measuring the conversion of E1 to E2. The xCELLigence System was used for monitoring of cell proliferation.
Results: We found a substantial increase of HSD17B1 mRNA and protein amount in NSCLC tissues compared with histopathologically unchanged tissues in the group of male patients. An overexpression of *HSD17B1* was associated with squamous cell carcinoma and with lung cancer stage 3A. We showed that 5-dAzaC induces DNA demethylation of *HSD17B1* promoter, leading to increased HSD17B1 mRNA levels and protein activity in NSCLC cells. It resulted in enhanced E2 production in both cell lines and supported the proliferation of Calu-1 cells but not A549 cells.
Conclusion: Increased expression of *HSD17B1* in NSCLC may contribute to an elevated intratissue level of E2 and consequently may support the development and spread of cancer.

1. Introduction

Lung cancer (LC) is one of the leading and increasing causes of death from malignant disorders among men and women worldwide [1]. Despite recent progress of new treatment methods, the outcome is still unsatisfactory and survival rates remain low.

Clinical classification distinguishes two major types of LC: non-small cell lung cancer (NSCLC), accounting for approximately 80% of all LCs, and small cell lung cancer (20%). NSCLCs are a heterogeneous group of carcinomas derived from epithelial cells, consisting mainly of three histological subtypes: adenocarcinoma (ADC), squamous cell carcinoma (SCC) and large cell carcinoma [2].

Over the past few decades, LC has been extensively studied in order to identify genes, signaling pathways and chromosomal rearrangements involved in an increased susceptibility to this disease. [3–10]. Apart from genetics, there are many identified environmental and lifestyle factors correlated with LC development. It is well known that the incidence of LC is connected with a history of smoking and cigarettes are still the main risk factor for this disease. However, recent studies provide novel insights into the contribution of sex steroid hormones in LC pathogenesis [11].

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Currently an upward trend in the incidence of NSCLC is observed among females [12]. Women are thought to be more vulnerable to carcinogens found in cigarette smoke and never smokers diagnosed with lung ADC are mostly females [12,13]. Young women suffer from tumors with rapid growth and more aggressive biology than postmenopausal counterparts [14]. The long-term use of hormone replacement therapy was positively correlated with a poor survival rate of LC patients [15,16]. Males diagnosed with NSCLC who presented high levels of E2 had significantly worse survival compared to men with lower E2 concentration [17]. Collectively, these evidences suggest that estrogens and their signaling pathways play a role in NSCLC pathogenesis, but it still remains uncertain.

Estrogens exert their effects on target tissues by estrogen receptors (ERs) via genomic and non-genomic pathways. The classical, genomic signaling involves 17-beta-estradiol (E2) binding to ERα or Erβ [18]. This ligand-dependend activation leads to transcriptional regulation of target genes expression [19]. Rapid cellular response via membrane-associated receptors is referred as non-genomic actions [20]. Consequently, sex steroid hormones promote the enzymatic modification of signaling molecules, increase the level of second messengers and activate various kinases [21].

Though there have been conflicting reports of the presence of both ERs in lung tumors, a number of studies demonstrated that at least ERβ is expressed in most human NSCLC cell lines, tumors and cells derived from normal lung tissue, regardless of gender [22–27]. Either genomic or non-genomic actions of estrogens were confirmed during normal lung development and in pulmonary disorders [21,27,28]. Previous investigations revealed that estrogens promote the growth of NSCLC cell lines through both pathways [21,23–27,29].

Although E2 is mainly produced in the ovaries, it can also be locally synthesized in peripheral tissues at both genders [30]. The 17-beta-hydroxysteroid dehydrogenase type 1 (HSD17B1) enzyme catalyzes the reduction of estrone (E1) to the most potent estrogen, E2 [31]. Thus, HSD17B1 plays a pivotal role in the regulation of E2 metabolism in extragonadal tissues. Recent studies indicated that androgens and estrogens are metabolized within the lung [14]. However, little is known about *HSD17B1* expression and its correlation between disturbances in the ratio of E2/E1 in NSCLC tumor milieu.

In this study we examined the status of HSD17B1 in lung cancerous and corresponding histopathologically unchanged tissues obtained from patients with NSCLC, at both mRNA and protein levels. We also investigated the association between *HSD17B1* expression and clinicopathological features of patients with NSCLC. Than we employed A549 and Calu-1 cell lines to examine the biological significance of HSD17B1 in NSCLC in vitro.

2. Materials and methods

2.1. Antibodies and reagents

All antibodies and reagents are described in Supplementary Data 1.

2.2. Patient material

Primary lung cancerous tissues were obtained between March and December 2012 from 48 patients with NSCLC who underwent surgical resection at the Department of Thoracic Surgery, Poznan University of Medical Sciences, Poland (Tables 1 and 2, and Supplementary Table 1). Patients included in this study did not receive chemotherapy and/or radiation therapy before surgery. Histopathologically unchanged lung tissue, located at least 10–20 cm away from the cancerous lesions, was obtained from the

Table 1
Differences in HSD17B1 transcript and protein levels in lung cancer tissues and histopathologically unchanged tissues from patients with NSCLC including gender, patient age and histologic type of NSCLC.

Variables	Number of cases	HSD17B1 transcript			HSD17B1 protein		
		Cancerous tissues Median (range)	Histopathologically unchanged tissues Median (range)	*p* value HSD17B1 mRNA	Cancerous tissues Median (range)	Histopathologically unchanged tissues MEDIAN (range)	*p* value HSD17B1 protein
Total no. of patients	48	2.948 (2.016–3.704)	2.607 (2.009–3.404)	**0.00138**	2.454 (1.423–3.977)	2.227 (0.770–3.478)	**0.000801**
Gender							
Male	35	3.040 (2.230–3.704)	2.606 (2.009–3.404)	**0.000325**	2.464 (1.423–3.487)	2.166 (0.770–2.986)	**0.000925**
Female	13	2.556 (2.016–3.425)	2.615 (2.450–2.940)	0.758	2.423 (2.062–3.977)	2.300 (1.775–3.478)	0.238
Patient age							
≤60	15	2.638 (2.341–3.703)	2.592 (2.449–2.944)	0.507	2.511 (2.256–3.977)	2.354 (1.567–3.478)	0.213
>60	33	3.040 (2.016–3.704)	2.607 (2.009–3.404)	**0.000937**	2.443 (1.423–3.487)	2.144 (0.780–2.915)	**0.002**
Histologic type							
Adenocarcinoma	26	2.670 (2.016–3.704)	2.610 (2.329–3.404)	0.346	2.417 (2.062–3.977)	2.254 (1.775–3.478)	**0.0101**
Squamous cell carcinoma	22	3.044 (2.341–3.703)	2.600 (2.009–3.182)	**0.000287**	2.474 (1.423–3.487)	2.011 (0.800–2.986)	**0.0172**
Histologic type and Gender							
adenocarcinoma, male	13	2.711 (2.230–3.704)	2.606 (2.329–3.404)	0.259	2.343 (2.172–3.009)	2.208 (1.806–2.541)	**0.0120**
Adenocarcinoma, female	13	2.556 (2.016–3.425)	2.615 (2.449–2.940)	0.758	2.423 (2.062–3.977)	2.300 (1.775–3.478)	0.238
Squamous cell carcinoma, male	22	3.044 (2.341–3.703)	2.600 (2.009–3182)	**0.000287**	2.474 (1.423–3.487)	2.011 (0.800–2.986)	**0.0172**
Squamous cell carcinoma, female	–	–	–	–	–	–	–

The HSD17B1 transcript levels were standardized by the geometric mean of PBGD and hMRPL19 cDNA levels and expressed as decimal logarithm of multiples of these cDNA copies in the calibrator. The amount of Western blot detected proteins was presented as the decimal logarithm of HSD17B1 to GAPDH band optical density ratio. Statistically significant results are indicated as bolded *p* value. The normality of the observed patient data distribution was assessed by Shapiro–Wilk test and *U*-Mann–Whitney test was used to compare the mean values. $p < 0.05$ was considered as statistically significant.