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GASC1 expression in lung carcinoma is associated with smoking and prognosis of squamous cell carcinoma

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Summary. GASC1 (gene amplified in squamous cell carcinoma 1) encodes a nuclear protein that epigenetically catalyses the lysine demethylation of histones.

We investigated the expression of GASC1 in different histological subtypes of lung cancer (n=289). Percentage value of GASC1 immunohistochemical expression was evaluated separately in the nuclei and cytoplasms of epithelial cancer cells. The results were compared with clinicopathologic factors and the smoking history of the patients.

In lung tumor cells, 38% of nuclei and 54% of the cytoplasms stained positive for GASC1. Adenocarcinomas expressed more GASC1 nuclear (p=0.00011) and cytoplasmic (p=0.00074) positivity than squamous cell carcinoma. Smokers displayed less nuclear and cytoplasmic GASC1 expression than non-smokers (p=0.028 and p=0.036, respectively). Similarly, patients with more cytoplasmic positive staining had fewer pack years (p=0.043). Nuclear GASC1 expression had an impairing effect on survival when all histological lung cancer types were analysed together (p=0.039) and separately in squamous cell lung carcinoma (p=0.016).

The results reveal that GASC1 expression is higher in adenocarcinoma than squamous cell carcinoma. Smoking decreases GASC1 expression in tumor cells, indicating that tobacco smoke may influence the methylation of histone 3 lysine residues in lung cancer. Nonetheless, nuclear GASC1 predicts a poor prognosis, especially in squamous cell carcinoma.

Key words: GASC1, Epigenetics, Histone demethylase, Lung cancer

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Introduction

GASC1 (gene amplified in squamous cell carcinoma 1, also known as KDM4C or JMJD2C) encodes a nuclear protein catalyzing lysine demethylation of histones. It is situated on the 9p23-24 amplicon and considered as one of this region's novel putative oncogenes (Yang et al., 2000). This histone demethylase contains a Jumonji C (JmjC) domain and it catalyzes histone demethylation of H3 lysine 9 (H3K9) and H3 lysine 36 (H3K36) (Cloos et al., 2006; Shi and Whetstine, 2007; Klose and Zhang, 2007; Whetstine et al., 2006), when H3K9 is mono- di- or trimethylated and H3K36 is di- or trimethylated (Yang et al., 2000; Shin and Janknecht, 2007). Demethylation of both H3K9 and H3K36 is linked to transcriptional activation (Steele-Perkins et al., 2001; Liu et al., 2009).

In human breast cancer, GASC1 is believed to be as a driving oncogene in the 9p23-24 amplicon (Liu et al., 2009). The expression level of its transcript is higher in aggressive basal-type breast cancers than non-basal-type (Liu et al., 2009). Basal breast cancer subtype is characterized by basal myoepithelial cell markers and is triple negative and often CK5/6+ or EGFR+ (Broeks et al., 2011). It also has transforming properties in vitro and its amplification and overexpression induces anchorage-

independent growth, growth factor independent proliferation and altered morphogenesis in three-dimensional cultures, as well as the formation of mammospheres (Liu et al., 2009). Knock down of GASC1 inhibits breast cancer cell proliferation (Liu et al., 2009). In addition, the demethylase activity of GASC1 regulates expression of genes critical for stem cell renewal, such as NOTCH1 (Liu et al., 2009).

GASC1's mRNA concentration, as determined by RT-PCR, is increased in prostate carcinoma as compared with normal tissue (Cloos et al., 2006). This regulates the function of the androgen receptor (AR) (Wissmann et al., 2007; Suikki et al., 2010) since its attachment to AR leads to demethylation of the repressing trimethylated H3K9 function and stimulation of AR-dependent target genes (Wissmann et al., 2007; Suikki et al., 2010). The oncogene GASC1 is also amplified and its expression is increased in medulloblastoma (Northcott et al., 2009). In mouse embryos, GASC1 is known to be expressed stage-specifically during preimplantational development displaying the highest activity from the two-cell to eight-cell stage (Wang et al., 2010). GASC1 is essential in early embryonal development and its depletion causes developmental arrest before the blastocyst stage (Wang et al., 2010). GASC1 regulates the pluripotency gene, NANOG, and its depletion evokes a significant down-regulation of NANOG, Pou5f1 and Sox1, as well as defects in stem cell self-renewal (Wang et al., 2010). In knock-down GASC1 embryos, the expression of oncogenes Klf4 and Myc is decreased, which might in part affect the cell cytokinesis (Wang et al., 2010).

GASC1 has been found to affect cancer development by regulating the function of the oncogene Mdm2 (Ishimura et al., 2009). Mdm2 is an E3 ubiquitin ligase of p53 which inhibits p53 activity in normal cells. Thus, over-expression of GASC1 increases Mdm2 expression through its demethylase activity since this reduces the basal level of p53 tumor suppressor protein (Ishimura et al., 2009). More studies are needed to clarify the functions of GASC1.

GASC1 expression has not been studied in lung carcinoma. In order to elucidate the role of GASC1 in lung cancer, we investigated the immunohistochemical expression of GASC1 in a large set of primary lung tumors and compared its expression to tumour histology and the clinical data of the patients. Additionally, we assessed the expression of GASC1 to known smoking habits and survival of the patients.

Materials and methods

Material

The tumors specimens being examined were retrieved from the archives of Department of Pathology, University of Oulu and had been collected during the years 1991- 1996. Our data contained lung carcinoma samples of 289 patients. Primary lung tumors consisted of squamous cell carcinomas (n=128), adenocarcinomas (n=118), small cell carcinomas (n=11), large cell carcinomas (n=12), carcinoid tumors (n=6) and adenosquamous carcinomas (n=14) (Table 1). There were 107 smokers (36.9%), 142 who had stopped smoking (49.0%) and 32 non-smokers (11.0%). The smoking information of nine patients (3.1%) was missing. The mean age of the patients was 63.8 years and median 65.5 years. This study had received permission from the ethical committee of the University Hospitals of Oulu and Kuopio.

Diagnoses had been determined according to the guidelines of the WHO international classification of lung and pleural tumors (Travis et al., 2004). Pack years were defined in the following manner: during their preoperative pulmonary function testing the patients were asked about their smoking history. The answers were registered as follows: the age which they began smoking, at the age which they had quit, possible breaks and the number of cigarettes smoked per day. Twenty cigarettes per day for a year is equal to one pack year.

Tissue microarray and immunohistochemistry

The samples were evaluated as tissue microarrays (TMAs). They were fixed in formalin and embedded in paraffin and two tumor regions were chosen from each sample. TMAs (diameter 1.3 mm) were incorporated using tissue microarrayer I device (Beecher Instruments, Silver Spring, MD, USA). TMAs in paraffin-embedded block were sliced into four micrometer thick sections which were then stained immunohistochemically. The sections were deparaffinated and rehydrated, then heated in a microwave oven for two times five minutes in a Tris-EDTA buffer (pH=9.0), incubated in a Tris-EDTA buffer for 20 minutes and washed twice for five minutes in phosphate buffered saline (PBS). Endogenous peroxide was blocked with 5% hydrogen peroxide (5 minutes), then washed twice in water for five minutes and twice in PBS for five minutes. Non-specific binding was blocked with 1.5% normal serum in PBS for 25 minutes at room temperature. The sections were incubated overnight in +4 degrees Celsius in KDM4C monoclonal antibody (clone 4B1, Novus Biologicals), dilution 1:100. Negative controls were not prepared with

Table 1. The frequencies of histological types of lung carcinoma in our data.

	frequency
Squamous cell carcinoma	128
Adenocarcinoma	118
Small cell carcinoma	11
Large cell carcinoma	12
Carcinoid tumors	6
Adenosquamous carcinoma	14
in total	289

primary antibody, only with 1% BSA dilution buffer. Samples were washed with PBS for two times five minutes, slides were incubated with a biotinylated secondary antibody (ABC Vectastain Elite Kit, Vector Laboratories, Burlingame, CA, USA) for 35 minutes. The sections were washed in PBS for two times five minutes. Two drops of ABC reagent solution were applied on slides and incubated under a coverglass for 45 minutes. The slides were washed with PBS twice for five minutes. The colour was developed with DAP (diaminobenzidine tetrahydrochloride, 3 minutes) (Sigma, St. Louis, MO, USA). The slides were counterstained with Mayer hematoxylin (4 minutes), washed, dehydrated, cleared and mounted with Depex (BDH, Poole, UK). A breast cancer sample, which was known to express GASC1, was used as a positive control.

Evaluation of samples

All samples were evaluated twice with a microscope (KU, YS). First, the percentage of GASC1 positively stained nuclei and cytoplasm of epithelial tumor cells were evaluated separately according to location, dividing them into four groups: 0-5%=(0), from over 5 to 30%=(1), from over 30 to 60%=(2) and from over 60 to 100%=(3). To simplify statistical analysis, the cases were then divided into two groups; 0-5% nuclear expression=negative, over 5% positivity=positive. Cytoplasmic expression was divided into two groups according to the median expression of the cases.

Statistical analyses

The data were analysed statistically using SPSS 17.0 for Windows software (SPSS Inc., Chicago Ill.). Pearson's Chi square test, Fischer's Exact test (2x2 contingency), t-test of independent groups in analyzing pack years, Cox-Regression for multivariate analyses, Kaplan-Meier's graph in analyzing survival, Log Rank, Tarone-Ware and Breslow statistical analyses were used as statistical methods. P-values under 0.05 were considered significant.

Results

Staining frequencies of nuclei and cytoplasm of tumors

Positive GASC1 expression was present more often in the cytoplasms than in the nuclei. One hundred and sixty three (62.0%) tumor samples showed negative nuclear GASC1 expression and 100 (38.0%) samples showed positive GASC1 expression. 120 (45.5%) tumor samples showed negative cytoplasmic GASC1 expression and 143 (54.5%) samples presented positive GASC1 expression (Table 2, Figs. 1, 2).

Histological subtypes and their associations

One in four of squamous cell carcinomas displayed positive nuclear GASC1 expression and 45.8% exhibited positive cytoplasmic expression. Half of the adenocarcinomas expressed positive nuclear staining and 68.9% had positive cytoplasmic staining whereas only 18.2% of small cell carcinomas expressed both nuclear

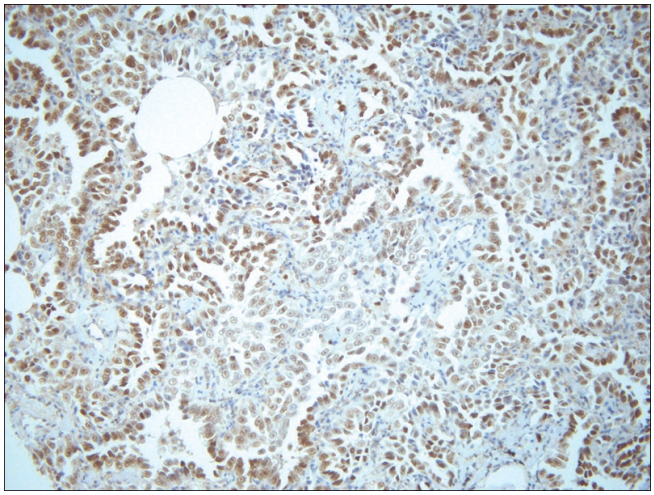

Fig. 1. Example of a case with nuclear positivity. The figure shows positively stained nuclei in lung adenocarcinoma cells.

Table 2. Nuclear and cytoplasmic GASC1 expression in different subtypes of lung carcinomas.

	Nuclear GASC1 expression			Cytoplasmic GASC1 expression		
	negative	positive	in total	negative	positive	in total
Squamous cell carcinoma	90 (25%)	30 (75%)	120	65 (54.2%)	55 (45.8%)	120
Adenocarcinoma	53 (50%)	53 (50%)	106	33 (31.1%)	73 (68.9%)	106
Small cell carcinoma	9 (81.8%)	2 (18.2%)	11	9 (81.8%)	2 (18.2%)	11
Large cell carcinoma	4 (44.4%)	5 (55.6%)	9	4 (44.4%)	5 (55,6%)	9
Carcinoid tumors	2 (60%)	3 (40%)	5	2 (60%)	3 (40%)	5
Adenosquamous carcinoma	5 (41.7%)	7 (58.3%)	12	7 (58.3%)	5 (41,7%)	12
in total	163	100	263	120	143	263

and cytoplasmic GASC1 expression. Large cell carcinomas expressed equally levels of nuclear and cytoplasmic GASC1 protein (55.6%), as did carcinoid tumors (60.0%). The majority (58.3%) of adenosquamous carcinomas displayed positive nuclear GASC1 expression and 41.7% exhibited positive cytoplasmic GASC1 expression (Tables 2, 3).

Adenocarcinomas displayed more nuclear GASC1 expression than squamous cell carcinomas (p=0.00011). Adenosquamous carcinomas also displayed more nuclear GASC1 expression than squamous cell carcinomas (p=0.037). Adenocarcinomas expressed more cytoplasmic GASC1 than squamous cell carcinomas or small cell carcinomas (p=0.00074 for former, p=0.0016 for latter) (Table 3).

pT, pN, pM

There was no association between the GASC1 expression in tumor cell nuclei or cytoplasms and tumor size, metastases in regional lymph nodes or distant metastases (TNM staging). The expression was not associated with histological malignancy of the tumor (grading). Analyzing separately squamous cell lung carcinomas and adenocarcinomas, no statistical association was found in this respect (Table 4).

Table 3. The associations between GASC1 expression and different histological subtypes.

Histological subtype	Nuclear GASC1 expression		Fisher's exact test (two-sided p-value)
	0 n (%)	1 n (%)	
Squamous cell carcinoma	90 (75.0)	30 (25.0)	0.00011
Adenocarcinoma	53 (50.0)	53 (50.0)	
Squamous cell carcinoma	90 (75.0)	30 (75.0)	0.037
Adenosquamous carcinoma	5 (41.7)	7 (58.3)	
	Cytoplasmic GASC1 expression		
	0 n (%)	1 n (%)	
Squamous cell carcinoma	65 (54.3)	55 (45.8)	0.00074
Adenocarcinoma	33 (31.1)	73 (68.9)	
Small cell carcinoma	9 (81.8)	2 (18.2)	0.0016
Adenocarcinoma	33 (31.1)	73 (68.9)	

Table 4. GASC1 expession and clinicopathilogic factors in squamous cell carcinoma and adenocarcinoma.

	Nuclear GASC1 expression				Cytoplasmic GASC1 expression			
	Squamous cell carcinoma		Adenocarcinoma		Squamous cell carcinoma		Adenocarcinoma	
	0 n (%)	1 n (%)	0 n (%)	1 n (%)	0 n (%)	1 n (%)	0 n (%)	1 n (%)
pT								
1	14 (13.0)	4 (3.7)	13 (13.7)	9 (9.5)	10 (9.3)	8 (7.4)	7 (7.4)	15 (15.8)
2	49 (45.4)	20 (18.5)	26 (27.4)	33 (34.7)	39 (36.1)	30 (27.8)	19 (20.0)	40 (42.1)
3	13 (12.0)	4 (3.7)	7 (7.4)	3 (3.2)	9 (8.3)	8 (7.4)	1 (1.1)	9 (9.5)
4	3 (2.8)	1 (0.9)	2 (2.1)	2 (2.1)	2 (1.9)	2 (1.9)	3 (3.2)	1 (1.1)
pN								
0	39 (36.8)	14 (13.2)	25 (26.9)	27 (29.0)	32 (30.2)	21 (19.8)	14 (15.1)	38 (40.9)
1	25 (23.6)	13 (12.3)	11 (11.8)	11 (11.8)	20 (18.9)	18 (17.0)	10 (10.8)	12 (12.9)
2	12 (11.3)	2 (1.9)	10 (10.8)	7 (7.5)	8 (7.5)	6 (5.7)	5 (5.4)	12 (12.9)
3	1 (0.9)	0 (0)	0 (0)	2 (2.2)	0 (0)	1 (0.9)	0 (0)	2 (2.2)
pM								
0	75 (70.8)	28 (26.4)	44 (46.8)	43 (45.7)	57 (53.8)	46 (43.4)	26 (27.7)	61 (64.9)
1	2 (1.8)	1 (0.9)	3 (3.2)	4 (4.3)	3 (2.8)	0 (0)	4 (4.3)	3 (3.2)
grade								
I	10 (8.3)	3 (2.5)	6 (5.7)	7 (6.6)	8 (6.6)	5 (4.1)	5 (4.7)	8 (7.5)
II	46 (38.0)	11 (9.1)	23 (21.7)	23 (21.7)	35 (28.9)	22 (18.2)	13 (12.3)	33 (31.3)
III	23 (19.0)	14 (11.6)	14 (13.2)	13 (12.3)	14 (11.6)	23 (19.0)	13 (12.3)	14 (13.2)
IV	0 (0)	1 (0.8)	0 (0)	1 (0.9)	1 (0.8)	0 (0)	0 (0)	1 (0.9)
m	11 (9.1)	2 (1.7)	10 (9.4)	9 (8.4)	7 (5.8)	6 (5.0)	2 (1.9)	17 (16.0)
stage								
I	19 (15.7)	6 (5.0)	1 (0.9)	12 (11.3)	16 (13.2)	9 (7.4)	4 (3.8)	9 (8.5)
II	6 (5.0)	6 (5.0)	6 (5.9)	4 (3.8)	7 (5.8)	5 (4.1)	5 (4.7)	5 (2.7)
III	8 (6.6)	1 (0.8)	5 (4.7)	2 (1.8)	5 (4.1)	4 (3.4)	1 (0.9)	6 (5.7)
IV	1 (0.8)	1 (0.8)	1 (0.9)	0 (0)	1 (0.8)	1 (0.8)	0 (0)	1 (0.9)
m	56 (46.3)	17 (14.0)	40 (37.7)	35 (33.0)	36 (29.8)	37 (30.6)	23 (21.7)	52 (49.1)
cause of death, n								
lung carcinoma (%)	47 (39.2)	23 (19.2)	39 (36.8)	33 (31.1)	37 (30.8)	33 (27.5)	20 (18.9)	53 (49.1)
other (%)	43 (35.8)	7 (5.8)	14 (13.2)	20 (18.9)	28 (23.4)	22 (18.3)	13 (12.2)	21 (19.8)

m, missing.

Tobacco smoking

Non-smokers had more nuclear GASC1 positivity than smokers (p=0.028) (Table 5). This was also seen when comparing nuclear GASC1 expression of smokers to ex-smokers, together with non-smokers (p=0.034). Analyzing only squamous cell carcinomas, there was an association between smokers and ex-smokers as well as smokers and ex-smokers together with non-smokers (p=0.019 the former, p=0.031 the latter): smokers expressed less nuclear GASC1 in both cases.

Cytoplasmic GASC1 expression and smoking status correlated with each other (p=0.032). Non-smokers and ex-smokers expressed more cytoplasmic GASC1 positivity than smokers (p=0.036, p=0.015, respectively) (Table 5). In squamous cell carcinomas, ex-smokers had more cytoplasmic GASC1 expression than smokers (p=0.00001). Furthermore, smokers exhibited less cytoplasmic GASC1 positivity than in the ex-smokers' and non-smokers' group (p=0.000006). Those who had started smoking later had more often GASC1 negative tumors, pack years or duration of smoking did not associate with GASC1 expression.

Survival

Nuclear GASC1 expression associated with survival when the patient had died of lung carcinoma. In Cox regression multivariate analysis nuclear GASC1 expression was an independent prognostic factor (p=0.015). Other factors which posessed independent prognostic value were lymph node metastasis (p=0.035) and distant metastasis (p=0.036). Based on our results, nuclear GASC1 positivity showed nearly a statistically significant impairing effect on survival (Log Rank=0.073, Breslow=0.039, Tarone-Ware=0.052). Cytoplasmic GASC1 expression was not associated with survival (Log Rank=0.19). Separately, in squamous cell carcinomas, an association existed between nuclear positivity and survival (Log Rank=0.016, Breslow=0.036, Tarone-Ware=0.026): patients with nuclear GASC1 expression had poorer survival rates compared with those whose tumors did not express this protein (Fig. 3). Cytoplasmic GASC1 expression of squamous cell carcinomas did not associate with survival (Log Rank=0.75). If only adenocarcinomas were studied, no

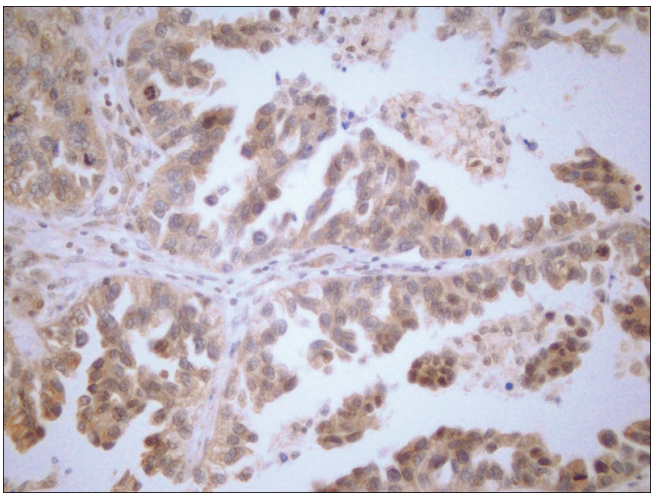

Fig. 2. Example of a case with cytoplasmic positivity. Cytoplasmic positivity can be seen in a case of papillary lung adenocarcinoma.

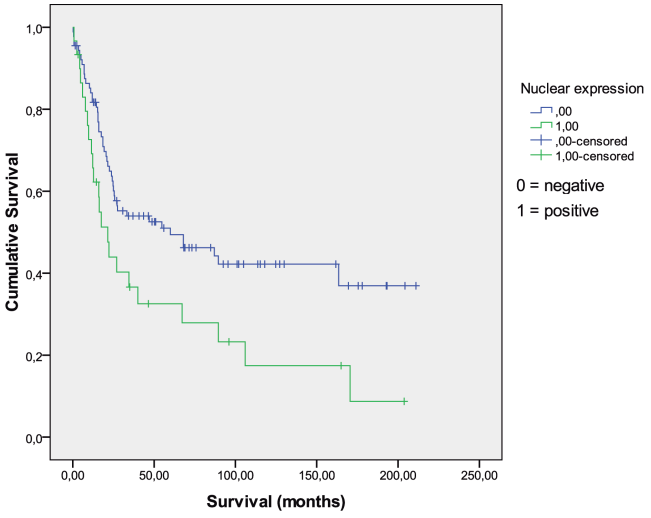

Fig. 3. The association of nuclear GASC1 expression and survival in squamous cell lung carcinoma (Log Rank=0.016, Breslow=0.036, Tarone-Ware=0.026).

Table 5. The association between nuclear and cytoplasmic GASC1 expression and smoking status.

	Nuclear GASC1 expression		Fisher's exact test	Cytoplasmic GASC1 expression		Fisher's exact test
	negative n (%)	positive n (%)	(two-sided p-value)	negative n (%)	positive n (%)	(two-sided p-value)
Smoker	68 (70.1)	29 (29.9)	0.028	55 (56.7)	42 (43.3)	0.036
Non-smoker	14 (46.7)	16 (53.3)		10 (33.3)	20 (66.7)	

(smoker, non-smoker) (p=0.028 the former, p=0.036 the latter)

association was found between nuclear or cytoplasmic GASC1 expression and survival (Log Rank=0.84 for nuclei, Log Rank=0.20 for cytoplasms).

Discussion

This is the first study to demonstrate that adenocarcinomas of the lung expressed more GASC1 protein in both nuclei and cytoplasms of tumor cells than squamous cell lung carcinomas. Adenosquamous carcinomas also expressed more nuclear GASC1 than squamous cell carcinomas. Furhermore, GASC1 expression was associated with tobacco smoking. The lung carcinoma tumors of tobacco smokers expressed less nuclear and cytoplasmic GASC1 protein than those of non-smokers. In particular, in squamous cell carcinomas, a difference existed between smokers and non-smokers or ex-smokers' cytoplasmic expression of GASC1 protein with smokers having less GASC1 expression than the group of non-smokers and ex-smokers. Pack years were also associated with GASC1 expression: patients with less pack years expressed more cytoplasmic GASC1 protein. Thirdly, GASC1 expression was associated with survival. Positive nuclear GASC1 expression had an impairing effect on survival in squamous cell carcinoma but not in adenocarcinoma.

The existing data on GASC1 protein in lung cancer is very limited. Initially, GASC1 enzyme was discovered in esophageal squamous cell carcinoma where it was amplified and over-expressed in several cell lines (Yang et al., 2000). In different cell lines of esophageal squamous cell carcinoma, GASC1 concentration was increased 3-5 fold compared with other cancer types and normal human fibroblasts (Yang et al., 2000). Inhibition of its expression decreases proliferation of tumor cells (Cloos et al., 2006). In a study of cancers of the upper aerodigestive tract [UADT] two variants of GASC1 [in SNPs] were strongly associated with increased risk of UADT cancer (Canova et al., 2009). In addition, in breast, prostate cancer and medulloblastoma, GASC1 is amplified and/or over expressed (Yang et al., 2000; Liu et al., 2009; Northcott et al., 2009).

In our study, GASC1 expression appeared more frequently in adenocarcinoma than in squamous cell carcinoma. It is interesting to note, based on our results, that the expression of GASC1 in adenocarcinoma did not associate with survival. Consequently, the tumor cells of adenocarcinoma have divergent qualities than those in squamous cell carcinoma, leading to a different GASC1 expression.

GASC1 protein expression was found in both nuclei and cytoplasms of lung carcinoma cells. GASC1 protein is active in the nuclei of cells where it covalently remodels histones. It is involved in carcinogenesis by regulating the function of Mdm2 gene (Ishimura et al., 2009). The overexpression of GASC1 leads to increased expression of Mdm2 ubiquitine ligase through its demethylase activity, which in turn leads to a reduction of the basal level of tumor suppressor protein p53 (Ishimura et al., 2009). Our results reveal that nuclear GASC1 expression of tumor epithelial cells has a promoting effect on lung carcinoma spread. This suggests that GASC1 is an oncogene and its function is somehow involved in deteriorated prognosis. In lung cancer carcinogenesis, the mutations in TP53 gene play a pivotal role in all lung cancer subtypes, especially in squamous cell carcinoma (Scoccianti et al., 2012). However, cytoplasmic GASC1 expression of tumor cells was not associated with survival in our study. Cytoplasmic GASC1 protein is likely to be inactive, stored or awaiting degradation and it does not seem to exert any functional or prognostic significance.

In the whole material of lung tumors, smokers displayed less GASC1 protein in both nuclei and cytoplasms than non-smokers. The differences in GASC1 expression were especially significant in squamous cell lung carcinomas, where ex-smokers also expressed more cytoplasmic GASC1 positivity than smokers. Pack years were inversely associated with survival: those with fewer pack years had more GASC1 expression in tumor cell cytoplasm. Thus, tobacco smoking seems to reduce or inhibit the expression of GASC1 in tumor cells and in this way GASC1 may be possibly involved in the epigenetic effects of smoking. Nevertheless, the modification is the opposite of what one might expect. The expression of GASC1 enhances Mdm2 expression and this in turn leads to enhanced degradation of p53 protein (Ishimura et al., 2009). Our findings reveal that tobacco smoke reduces GASC1 expression. The net impact of tobacco smoke is, however, hard to evaluate, since demethylation of histones may inactivate genes that are tumor promoting as well as tumor suppressor genes. Additionally, other carcinogenetic effects of tobacco smoke surely play an important role in this toxic effect. However, our finding suggests that tobacco smoke can influence epigenetic mechanisms of cells, thus demonstrating a link between environmental factors and DNA regulation mechanisms.

In the whole material of lung tumors, positive nuclear GASC1 expression had an impairing effect on survival. In squamous cell lung carcinoma, this stood out in contrast to adenocarcinoma, where no statistical association was found. The finding suggests that GASC1 activates/inactivates tumor promoting genes, especially in squamous cell carcinoma. It is known that squamous and adenocarcinomas display a different type of oncogenic progression. In adenocarcinomas, there are EGRF (Mitsudomi et al., 2006) and RAS (Kan et al., 2010) mutations as well as EML4-ALK translocations (Pillai and Ramalingam, 2012), whereas squamous cell carcinomas tend to exhibit BAI3, FBXW7, GRM8, ERBB4, MUC1b and RUNX1T1 mutations (Kan et al., 2010). GASC1 protein is active in the nucleus where it remodels histones covalently leading to decreased expression of tumor suppressor p53 and thus further on to carcinogenesis (Ishimura et al., 2009). The accumulation of epigenetic alterations can lead to a neoplastic phenotype. It is thus not surprising that the

nuclear expression of this protein exerts a detrimental impact on survival.

In conclusion, the study shows that both tumor histology and smoking status are associated with GASC1 expression. The former association may be explained by the fact that different cancer genes are activated in adenocarcinoma and squamous cell carcinoma, and GASC1 induced demethylation of histone proteins may influence these genes in a different way in the two types of tumors. Tobacco smoke decreases GASC1 expression suggesting that external carcinogens may influence the epigenetics of lung tumors.

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References

Broeks A., Schmidt M.K., Sherman M.E., Couch F.J., Hopper J.L., Dite G.S., Apicella C., Smith L.D., Hammet F., Southey M.C., Van 't Veer L.J., de Groot R., Smit V.T., Fasching P.A., Beckmann M.W., Jud S., Ekici A.B., Hartmann A., Hein A., Schulz-Wendtland R., Burwinkel B., Marme F., Schneeweiss A., Sinn H.P., Sohn C., Tchatchou S., Bojesen S.E., Nordestgaard B.G., Flyger H., Ørsted D.D., Kaur-Knudsen D., Milne R.L., Pérez J.I., Zamora P., Rodríguez P.M., Benítez J., Brauch H., Justenhoven C., Ko Y.D.; Genica Network, Hamann U., Fischer H.P., Brüning T., Pesch B., Chang-Claude J., Wang-Gohrke S., Bremer M., Karstens J.H., Hillemanns P., Dörk T., Nevanlinna H.A., Heikkinen T., Heikkilä P., Blomqvist C., Aittomäki K., Aaltonen K., Lindblom A., Margolin S., Mannermaa A., Kosma V.M., Kauppinen J.M., Kataja V., Auvinen P., Eskelinen M., Soini Y., Chenevix-Trench G., Spurdle A.B., Beesley J., Chen X., Holland H.; kConFab; AOCS, Lambrechts D., Claes B., Vandorpe T., Neven P., Wildiers H., Flesch-Janys D., Hein R., Löning T., Kosel M., Fredericksen Z.S., Wang X., Giles G.G., Baglietto L., Severi G., McLean C., Haiman C.A., Henderson B.E., Le Marchand L., Kolonel L.N., Alnæs G.G., Kristensen V., Børresen-Dale A.L., Hunter D.J., Hankinson S.E., Andrulis I.L., Mulligan A.M., O'Malley F.P., Devilee P., Huijts P.E., Tollenaar R.A., Van Asperen C.J., Seynaeve C.S., Chanock S.J., Lissowska J., Brinton L., Peplonska B., Figueroa J., Yang X.R., Hooning M.J., Hollestelle A., Oldenburg R.A., Jager A., Kriege M., Ozturk B., van Leenders G.J., Hall P., Czene K., Humphreys K., Liu J., Cox A., Connley D., Cramp H.E., Cross S.S., Balasubramanian S.P., Reed M.W., Dunning A.M., Easton D.F., Humphreys M.K., Caldas C., Blows F., Driver K., Provenzano E., Lubinski J., Jakubowska A., Huzarski T., Byrski T., Cybulski C., Gorski B., Gronwald J., Brennan P., Sangrajrang S., Gaborieau V., Shen C.Y., Hsiung C.N., Yu J.C., Chen S.T., Hsu G.C., Hou M.F., Huang C.S., Anton-Culver H., Ziogas A., Pharoah P.D. and Garcia-Closas M. (2011). Low penetrance breast cancer susceptibility loci are associated with specific breast tumor subtypes: findings from the Breast Cancer Association Consortium. Hum Mol Genet 20, 3289-3303.

Canova C., Hashibe M., Simonato L., Nelis M., Metspalu A., Lagiou P., Trichopoulos D., Ahrens W., Pigeot I., Merletti F., Richiardi L., Talamini R., Barzan L., Macfarlane G.J., Macfarlane T.V., Holcatova I., Bencko V., Benhamou S., Bouchardy C., Kjaerheim K., Lowry R., Agudo A., Castellsague X., Conway D.I., McKinney P.A., Znaor A., McCartan B.E., Healy C.M., Marron M. and Brennan P. (2009). Genetic associations of 115 polymorphisms with cancers of the upper aerodigestive tract across 10 European countries: the ARCAGE project. Cancer Res. 69, 2956-2965.

Cloos P.A., Christensen J., Agger K., Maiolica A., Rappsilber J., Antal T., Hansen K.H. and Helin K. (2006). The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. Nature 442, 307-311.

Ishimura A., Terashima M., Kimura H., Akagi K., Suzuki Y., Sugano S. and Suzuki T. (2009). Jmjd2c histone demethylase enhances the expression of Mdm2 oncogene. Biochem. Biophys. Res. Commun. 389, 366-371.

Kan Z., Jaiswal B.S., Stinson J., Janakiraman V., Bhatt D., Stern H.M., Yue P., Haverty P.M., Bourgon R., Zheng J., Moorhead M., Chaudhuri S., Tomsho L.P., Peters B.A., Pujara K., Cordes S., Davis D.P., Carlton V.E., Yuan W., Li L., Wang W., Eigenbrot C., Kaminker J.S., Eberhard D.A., Waring P., Schuster S.C., Modrusan Z., Zhang Z., Stokoe D., de Sauvage F.J., Faham M. and Seshagiri S. (2010). Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 466, 869-873.

Klose R.J. and Zhang Y. (2007). Regulation of histone methylation by demethylimination and demethylation. Nature Rev. Mol. Cell. Biol. 8, 307-318.

Liu G., Bollig-Fischer A., Kreike B., van de Vijver M.J., Abrams J., Ethier S.P. and Yang Z.Q. (2009). Genomic amplification and oncogenic properties of the GASC1 histone demethylase gene in breast cancer. Oncogene 28, 4491-4500.

Mitsudomi T., Kosaka T. and Yatabe Y. (2006). Biological and clinical implications of EGFR mutations in lung cancer. Int. J. Clin. Oncol. 11, 190-198.

Northcott P.A., Nakahara Y., Wu X., Feuk L., Ellison D.W., Croul S., Mack S., Kongkham P.N., Peacock J., Dubuc A., Ra Y.S., Zilberberg K., McLeod J., Scherer S.W., Sunil Rao J., Eberhart C.G., Grajkowska W., Gillespie Y., Lach B., Grundy R., Pollack I.F., Hamilton R.L., Van Meter T., Carlotti C.G., Boop F., Bigner D., Gilbertson R.J., Rutka J.T. and Taylor M.D. (2009). Multiple recurrent genetic events converge on control of histone lysine methylation in medulloblastoma. Nat. Genet. 41, 465-472.

Pillai R.N. and Ramalingam S.S. (2012). The biology and clinical features of non-small cell lung cancers with EML4-ALK translocation. Cur. Oncol. Rep. 14, 105-110.

Scoccianti C., Vesin A., Martel G., Olivier M., Brambilla E., Timsit J.F., the EUELC Consortium, Tavecchio L., Brambilla C., Field J.K. and Hainaut P. (2012). Prognostic value of TP53, KRAS and EGFR mutations in Non-Small Cell Lung Cancer: EUELC cohort. Eur. Respir. J. 40, 177-184

Shi Y. and Whetstine J.R. (2007). Dynamic regulation of histone lysine methylation by demethylases. Mol. Cell 25, 1-14.

Shin S. and Janknecht R. (2007). Diversity within the JMJD2 histone demethylase family. Biochem. Biophys. Res. Commun. 353, 973-977.

Steele-Perkins G., Fang W., Yang X.H., Van Gele M., Carling T., Gu J., Buyse I.M., Fletcher J.A., Liu J., Bronson R., Chadwick R.B., de la Chapelle A., Zhang X., Speleman F. and Huang S. (2001). Tumor formation and inactivation of RIZ1, an Rb-binding member of a nuclear protein-methyltransferase superfamily. Genes Dev. 15, 2250-2262.

Suikki H.E., Kujala P.M., Tammela T.L., van Weerden W.M., Vessella R.L. and Visakorpi T. (2010). Genetic alterations and changes in expression of histone demethylases in prostate cancer. Prostate 70, 889-898

Travis W.D., Brambilla E., Muller-Hermelink H.K. and Harris C.C. (2004). Tumours of the lung, pleura, thymus and heart. Pathology & genetics. WHO classification of tumours, IARC Press, Lyon.

Wang J., Zhang M., Zhang Y., Kou Z., Han Z., Chen D.Y., Sun Q.Y. and Gao S. (2010). The histone demethylase JMJD2C is stage-specifically expressed in preimplantation mouse embryos and is required for embryonic development. Biol. Reprod. 82, 105-111.

Whetstine J.R., Nottke A., Lan F., Huarte M., Smolikov S., Chen Z., Spooner E., Li E., Zhang G., Colaiacovo M. and Shi Y. (2006). Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. Cell 125, 467-481.

Wissmann M., Yin N., Muller J.M., Greschik H., Fodor B.D., Jenuwein T., Vogler C., Schneider R., Günther T., Buettner R., Metzger E. and Schüle R. (2007). Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. Nat. Cell. Biol. 9, 347-53.

Yang Z.Q., Imoto I., Fukuda Y., Pimkhaokham A., Shimada Y., Imamura M., Sugano S., Nakamura Y. and Inazawa J. (2000). Identification of a novel gene, GASC1, within an amplicon at 9p23-24 frequently detected in esophageal cancer cell lines. Cancer Res. 60, 4735-4739.

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