

Decreased *FAM107A* Expression in Patients with Non-small Cell Lung Cancer

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Abstract

Lung cancer is the leading cause of cancer-related death in the world. Early detection, based on molecular markers, could decrease mortality from this disease. Tumor development is often associated with inactivation or loss of tumor suppressor genes (TSGs). The aim of the present study was to analyze the expression level of *FAM107A* gene, a TSG located in 3p21.1, in lung cancer tumors and in tumor adjacent normal lung samples. Promoter methylation status of *FAM107A* was evaluated as the potential mechanism of its epigenetic silencing. The relationship between gene mRNA expression and tumor staging, metastasis status, and non-small cell lung cancer (NSCLC) histopathological subtypes in 60 patients was analyzed. Total RNA was isolated from tissue samples and gene expression was assessed in qPCR assay. Gene promoter methylation status was evaluated in MSP reactions, using bisulfite converted DNA and two pairs of primers: methylated and unmethylated. We found that the expression of the gene was dramatically decreased in all NSCLC samples and was significantly lower than in tumor adjacent normal lung tissue. Promoter methylation of *FAM107A* gene was confirmed only in the minority of NSCLCs. The results highlight the importance of *FAM107A* in lung carcinogenesis, although indicate other than promoter hypermethylation mechanism of the gene decreased expression.

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Keywords

Epigenetic mechanisms • Gene expression • Lung cancer • NSCLC
• Promoter methylation

1 Introduction

Lung cancer remains the most common cancer in the world, both in term of cases (1.6 million cases, 12.7 % of total) and deaths (1.4 million deaths, 18.2 % of total), being the most common cancer in men worldwide (1.1 million cases, 16.5 % of total), and the fourth most frequent cancer in women (513,000 cases, 8.5 % of all cancers) (Ferlay et al. 2010). According to histopathological verification, lung cancer is classified into two major groups based on its biological phenotype, therapy, and prognosis: non-small cell lung cancer (NSCLC), including squamous cell carcinoma (SSC), adenocarcinoma (AC), and large cell carcinoma (LCC) which account for approximately 80 % of all primary lung cancers, and small cell lung cancer (SCLC) which constitutes about 20 % of malignant cases (Travis et al. 2004). Lung cancer is characterized by high fatality: the ratio of mortality to incidence is 0.86 (Ferlay et al. 2010), mainly because the disease is most often diagnosed at an advanced stage when there are few curative treatment options. The overall 5-year survival for NSCLC does not exceed 15 %, however, in stage I disease it increases up to 83 % (Kathuria et al. 2014). This highlights the importance of detecting lung cancer at an early and potentially treatable stage. It is believed that combining molecular information regarding biomarkers that are highly sensitive and specific with clinical risk features may offer the improved way to identify individuals with the highest risk for lung cancer development or for lung cancer progression.

The *FAM107A* gene, a member of the family with sequence similarity 107 (*FAM107*), also named *DRRI* (downregulated in renal cell carcinoma), and *TU3A* (Tohoku University cDNA clone A on chromosome 3), is localized in

chromosomal region 3p21.1, spans ~10 kb of genomic DNA, and the mature RNA (3.5 kb) encodes a 144-amino acid protein (Wang et al. 2000; Yamato et al. 1999). The protein has nuclear localization and contains a coiled-coil domain (Wang et al. 2000). Such structure suggests a role for *FAM107A* in regulating gene transcription. The potential mechanism could be *via* epigenetic regulation, as it interacts with transcriptional adaptor (Tada) 2 α , a protein that is part of the histone acetyltransferase (HAT) complex (Nakajima and Koizumi 2014). As found in another study, the gene is involved in cell cycle regulation *via* apoptosis induction (Liu et al. 2009). Although the mechanism is still unknown, it probably acts through indirect mechanism.

FAM107A is considered a tumor suppressor gene (TSG) due to its decreased expression in various types of cancer. The results of studies show no or decreased gene expression in renal cell carcinoma (RCC), ovarian cancer, prostate cancer, as well as in lung cancer cell lines (Liu et al. 2009; Zhao et al. 2007; Kholodnyuk et al. 2006; Wang et al. 2000). Thus, loss of expression of *FAM107A* can play a role in the development of epithelial neoplasms. On the other hand, the highly increased expression of *FAM107A* has been found in the invasive component of glioma and has been associated with tumor invasion (cell migration and expansion) *via* cytoskeleton modulation/rearrangements (Nakajima and Koizumi 2014).

The most frequent mechanisms of TSG inactivation is loss of heterozygosis (LOH) and mutation in the remaining allele or epigenetic silencing, due to promoter methylation. Allelic deletion at human short arm of chromosome 3 – encompassing *FAM107A* locus – occurs frequently in cancers, including lung tumors (Zabarovsky et al. 2002). As no mutations were

identified in *FAM107A*, the hypothesis of gene hypermethylation was tested in several studies. It has been confirmed in several primary cancers and cancer cell lines (Awakura et al. 2008; Vanaja et al. 2006; van den Boom et al. 2006).

Most studies performed to date have been carried out using lung cancer cell lines or only small groups of clinical cases. However, the positive results indicate the role of this gene in lung carcinogenesis. This encouraged us to analyze the expression level and methylation status of *FAM107A* in non-small cell lung cancer patients.

The pre-specified hypothesis tested in the study was that *FAM107A* expression level was decreased in primary non-small cell lung cancer with promoter hypermethylation as the responsible epigenetic mechanism of gene silencing. We tried to elucidate the role of *FAM107A* in early lung carcinogenesis and cancer progression.

2 Methods

The study was approved by the Ethics Committee of the Medical University of Lodz in Poland (permission no. RNN/140/10/KE). Written informed consent was obtained from each patient.

2.1 Characterization of NSCLC Tissue Samples and Patients Clinical Characteristics

Biological material (lung tissue) was obtained from 60 patients admitted to the Department of Thoracic Surgery, General and Oncologic Surgery, Medical University of Lodz in Poland, during July 2010–March 2013. Based on the results of preoperative cytological/histological assessment, the patients were qualified for surgery and were treated by either lobectomy or pneumectomy. Immediately after resection, lung tissue samples (100–150 mg) and the adjacent non-cancerous macroscopically unchanged tissues (100 mg; 10 cm distant from the primary lesion) obtained from the same patients were placed in a stabilization buffer RNAlater®

(Qiagen, Hilden, Germany). Each tissue sample was divided into smaller parts (30–50 mg) for individual analysis. All samples were frozen at -80°C .

The resected tissue specimens were post-operatively histopathologically evaluated and classified according to the AJCC staging (AJCC 2010) as well as TNM classification (pTNM) (Goldstraw et al. 2007; Mountain 1986). Histopathological assessments of tumor samples were obtained from pathomorphological reports, and were as follows: squamous cell carcinoma (SCC), adenocarcinoma (AC), and large cell carcinoma (LCC).

Histopathological verifications of NSCLC tissues are included in Table 1. The studied group consisted of 24 women and 36 men. All cases were primary tumors without chemo- or radiotherapy treatment. The smoking history was available for all patients and they were divided into groups according to their smoking habits: time of tobacco addiction and amount of cigarettes smoked.

2.2 RNA Extraction, Real-Time PCR (qPCR Method)

Total RNA was extracted from lung samples (cancer tissue obtained from the center of lung lesion and macroscopically unchanged lung tissue obtained from the most distant site from the resected lesion) using Universal RNA Purification Kit (Eurz, Gdansk, Poland) according to the manufacturer's recommendations. The qualitative and quantitative assessments of RNA samples were determined by minielectrophoresis in polyacrylamide gel using RNA 6000 Pico/Nano LabChip kit (Agilent 2100 Bioanalyzer; Agilent Technologies, Santa Clara, CA).

Complementary DNA (cDNA) was transcribed from 100 ng of total RNA, using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) in a total volume of 20 μl per reaction. Reverse transcription (RT) master mix contained: 10 \times RT buffer, 25 \times dNTP Mix (100 mM), 10 \times RT Random Primers, MultiScribe™ Reverse Transcriptase,

Table 1 Clinicopathological features of the studied NSCLC group

Patients' demographics	n = 60	
Women	Mean age: 63.1 ± 7.8 (years)	24 (40 %)
Men	Mean age: 65.8 ± 7.3 (years)	36 (60 %)
Tobacco addiction and consumption	n = 60	
The smoking period	Smokers	55 (92 %)
	< 40 years	26 (47 %)
	≥ 40 years	29 (53 %)
	Non smokers	5 (8 %)
The amount of cigarettes smoked	10–15 cigarettes per day	6 (11 %)
	20 cigarettes per day (1 pack)	39 (71 %)
	30–40 cigarettes per day (1.5–2 packs)	10 (18 %)
Pack Years ^a (PYs)	Up to 40 PYs	26 (47 %)
	≥40 PYs	29 (53 %)
Histopathological verifications of NSCLC samples	n = 60	
Histopathological type of NSCLC	Squamous cell carcinoma (SCC)	34 (57 %)
	Non-squamous cell carcinoma (NSCC)	26 (43 %)
	Adenocarcinoma (AC)	21 (35 %)
	Large cell carcinoma (LCC)	5 (8 %)
AJCC classification ^b	AJCC IA/IB	12 (20 %)
	AJCC IIA/IIB	21 (35 %)
	AJCC IIIA/IIIB	27 (45 %)
pTNM classification ^c	T1	12 (20 %)
	T2	33 (55 %)
	T3/T4	15 (25 %)

^aPYs were calculated according to the NCI Dictionary of Cancer Terms: 1 Pack Year is equal to 20 cigarettes smoked per day for 1 year (<http://www.cancer.gov/dictionary?CdrID=306510>)

^bAJCC (2010)

^cpTNM – post-operative Tumor Node Metastasis classification (Goldstraw et al. 2007; Mountain 1986)

RNase Inhibitor and nuclease-free water. RT reaction was performed in a Personal Thermocycler (Eppendorf, Hamburg, Germany) in the following conditions: 10 min at 25 °C, followed by 120 min at 37 °C, then the samples were heated to 85 °C for 5 s, and hold at 4 °C.

The relative expression of the *FAM107A* gene was assessed in qPCR reactions using Micro Fluidic Cards with pre-loaded selected assays: Hs00200376_m1 for the *FAM107A* (family with sequence similarity 107A) gene and Hs00382667_m1 for *ESD* (esterase D) as the reference gene. The PCR mixture contained: 50 µl cDNA (50 ng) and 50 µl TaqMan® Universal Master Mix (Applied Biosystems, Carlsbad, CA). TaqMan Array card was centrifuged twice for 1 min at 1,200 rpm to fill the wells with PCR mixture. Then, it was sealed and placed in a 7900HT Fast Real-Time PCR

System (Applied Biosystems, Carlsbad, CA). The PCR conditions were as follows: after initial incubation at 50 °C for 2 min and AmpliTaq Gold® DNA polymerase activation at 94.5 °C for 10 min, real-time PCR amplification was processed in 40 cycles of 30 s denaturation at 97 °C, followed by 1 min elongation step at 59.7 °C.

The relative expression of *FAM107A* in the studied samples was assessed using the comparative delta-delta C_T method (TaqMan Relative Quantification Assay software, Applied Biosystems, Carlsbad, CA) and presented as RQ value, adjusted to *ESD* expression level. RNA isolated from normal lung tissue (Human Lung Total RNA, Ambion®, Life Technologies, Carlsbad, CA) served as a calibrator sample. RNAs obtained from macroscopically unchanged lung tissues formed the control group.

2.3 DNA Extraction, Bisulfite Conversion and Methylation-Specific PCRs (MSP Method)

The extraction of genomic DNA from NSCLC specimens was performed using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The quality and quantity of isolated DNA was spectrophotometrically assessed (Eppendorf BioPhotometer™ plus, Eppendorf, Hamburg, Germany). DNA samples with a 260/280 nm ratio in the range 1.8–2.0 were considered as high quality and used in further analysis.

Methylation status of the *FAM107A* gene was assessed by methylation-specific polymerase chain reaction (MSP) using bisulfite converted DNA. Genomic DNA (1 µg) was modified with sodium bisulfite, using the CpGenome™ Turbo Bisulfide Modification Kit (CHEMICON International, Millipore, Temecula, CA), according to the manufacturer's protocol. Concentration and purity of the modified DNA was spectrophotometrically estimated at 260/280 nm in a biophotometer (Eppendorf BioPhotometer™ plus, Eppendorf, Hamburg, Germany). The conventional MSP method was performed according to Herman et al. (1996), with some modifications. Briefly, MSP was performed for each sodium bisulfite modified DNA sample using AmpliTaq Gold® 360 DNA Polymerase (Applied Biosystems, Carlsbad, CA). Amplifications were conducted in a total volume of 12.5 µl in a Thermocycler SureCycler 8800 (Agilent Technologies, Santa Clara, CA). MSP master mix contained: 1,000 ng DNA, 0.7 µM of each primer (Sigma-Aldrich, Poznan, Poland), 2.5 µM dNTPs mix, 2.5 µM MgCl₂, Hot Start AmpliTaq Gold® 360 Polymerase (5 U/µl), 10x

Universal PCR buffer and nuclease-free water. PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles involving denaturation at 95 °C for 45 s, annealing temperature – appropriate for a given primer (see Table 2) – for 45 s and elongation at 72 °C for 1 min; the final elongation step was done at 72 °C for 10 min.

The set of primers for the studied gene was flanking the 1 kb 5' region upstream from the translation start point. Primers for the methylation-specific PCR were designed according to the criteria described by Feltus et al. (2003). Primer sequences for the methylated and unmethylated *FAM107A* promoter regions are given in Table 2.

In each PCR reaction, positive and negative MSP controls were included. CpGenome Universal Methylated DNA (enzymatically methylated human male genomic DNA) served as a positive methylation control and CpGenome Universal Unmethylated DNA (human fetal cell line) was used as a negative control (Chemicon International, Millipore, Temecula, CA). Additionally, blank samples with nuclease-free water were used instead of DNA as a control for PCR contamination.

The MSP products were separated electrophoretically on 2 % agarose gel and their concentration (ng) of MSP products (U and M DNA alleles) was estimated spectrophotometrically, using DNA1000 LabChip Kit on Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Afterwards, the Methylation Index (MI) was assessed for each sample, using the following formula: peak height of methylated products/(peak height of methylated products + peak height of unmethylated product), $MI = (M)/(M + U)$.

Table 2 Characterization of MSP primers used in the study

MSP primers	Forward primer	Reverse primer	Product length (bp)	Annealing temperature (°C)
Methylated (M)	TGTTTTTTTATTTA GGGGTTTTTTAAC	GACTAAACTCGACT ACAACAACGAC	195	67.8
Unmethylated (U)	TTTTTATTTAGGGG TTTTTTAATGT	AACTAAACTCAACT ACAACAACAAC	191	67.3

2.4 Statistical Analysis

The Kruskal-Wallis and Mann-Whitney U tests were used to compare the levels of relative expression (RQ values) between NSCLC subtypes, i.e., SCC, AC, and LCC, shown in box and whisker plots. Spearman's rank correlation coefficient, Mann-Whitney U test, and Kruskal-Wallis test were performed in order to evaluate the relationship between the expression level of the studied gene and examined parameters (patients' characteristics: age, gender, history of smoking and tumor staging according to pTNM and AJCC classifications). The results of relative expression analysis (RQ values) are presented as means \pm SE and means \pm SD. The accepted level of statistical significance was estimated at $P < 0.05$. Statistica for Windows 10.0 program (StatSoft, Cracow, Poland) was applied for calculations.

3 Results

3.1 Relative Expression of *FAM107A*

The relative expression level of *FAM107A* in the studied tissue samples, determined using delta-delta C_T method, was expressed as RQ values adjusted to the expression of *ESD* (endogenous control) and in relation to the expression level of a calibrator (normal lung tissue), for which $RQ = 1$. The obtained *FAM107A* RQ values were correlated with histopathological NSCLC subtypes (SCC, AC, and LCC), tumor staging (pTNM and AJCC), patients' age, gender, and smoking history.

FAM107A expression was decreased (RQ value < 1) in all except one (AC) studied NSCLC samples ($>98\%$), with mean RQ value of 0.14 ± 0.38 . The value of $RQ < 0.5$ is considered significant. In macroscopically unchanged lung tissues (adjacent to lung tumors), the mean *FAM107A* expression was 1.65 ± 1.46 . The difference between those two groups was significant ($P = 0.0001$, Mann-Whitney U test) (Fig. 1).

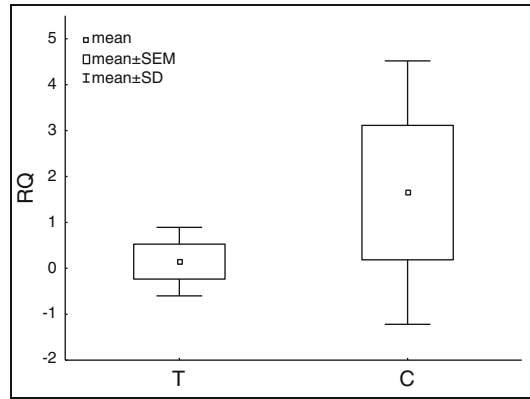


Fig. 1 Expression levels (mean RQ values) of the *FAM107A* gene in NSCLC (T, tumor) and macroscopically unchanged (C, control) tissue groups

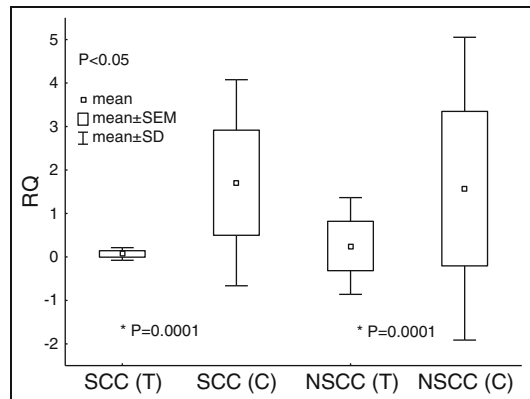


Fig. 2 Expression levels (mean RQ values) of the *FAM107A* gene in NSCLC histotypes (SCC and NSCC) vs. matching macroscopically unchanged tissue groups (Mann-Whitney U test)

Regarding the individual three NSCLC histotypes, *FAM107A* expression was not significantly different among them (SCC vs. AC vs. LCC: 0.07 vs. 0.24 vs. 0.27 ; $p = 0.36$, Kruskal-Wallis test). Due to a small number of cases in the LCC group, statistical analysis was also performed between two groups: SCC and NSCC (non-squamous cell carcinoma involving AC and LCC), but no statistically significant differences were noted (SCC vs. NSCC: 0.07 vs. 0.24 ; $p = 0.26$, Mann-Whitney U test). There were, however, differences in the *FAM107A* expression levels between the individual NSCLC histotypes (SCC or NSCC) and the matching macroscopically unchanged tissue groups as shown in Fig. 2.

There was no significant correlation between RQ values of *FAM107A* and the clinical features of NSCLC patients, i.e., patients' age (three age groups: ≤ 60 years, 60–70 years, over >70 years: 0.07 vs. 0.21 vs. 0.08; $p = 0.97$), gender (men vs. women: 0.09 vs. 0.22; $p = 0.65$), and smoking habit (current smokers vs. former smokers vs. never smokers: 0.10 vs. 0.12 vs. 0.56; $p = 0.88$), history of smoking assessed as PY (<25 PYs vs. 26–39 PYs vs. 40–45 PYs vs. ≥ 45 PYs: 0.23 vs. 0.12 vs. 0.07 vs. 0.14; $p = 0.67$), as well as histopathological features of tumor, i.e., pTNM classification (T1 vs. T2 vs. T3/T4: 0.05 vs. 0.18 vs. 0.14; $p = 0.97$), AJCC classification (IA/IB vs. IIA/IIB vs. IIIA/IIIB: 0.37 vs. 0.08 vs. 0.11; $p = 0.88$) (Kruskal-Wallis test, U Mann-Whitney's test followed by Spearman's rank correlation coefficient).

In the group of smokers (current and former, $n = 55$), the mean expression level of *FAM107A* was 0.07 in SCC and 0.16 in NSCC; however, no statistically significant difference was reached ($p = 0.39$, Mann-Whitney U test). Additionally, in the whole group of smokers, analysis was performed to find out if there was a correlation between gene expression and the amount of cigarettes smoked in relation to the length of the smoking (PYs). Spearman's rank correlation revealed $\rho = -0.17$, with no statistical significance ($p = 0.21$). Likewise, there was no significance in the individual NSCLC subtypes (SCC and NSCC) in smokers; ρ values were -0.17 and -0.16 , respectively ($p > 0.05$).

3.2 Methylation Status of *FAM107A*

Based on MSP results, the presence of both methylated alleles of *FAM107A* gene – representing 100 % methylation status (MI = 1) of the studied promoter region – was found only in 7 % of all studied cases, as presented in Table 3. Figure 3 illustrates the examples of totally methylated (MI = 1) and unmethylated (MI = 0) NSCLC samples.

Table 3 The presence of methylated (M) and unmethylated (U) alleles, expressed as MI, in the studied NSCLC groups

NSCLC subtype	MI = 1 (both M alleles)	MI = 0 (both U alleles)
SCC ($n = 31$)	3 (10 %)	28 (90 %)
AC ($n = 20$)	1 (5 %)	19 (95 %)
LCC ($n = 4$)	0 (0 %)	4 (100 %)
Total ($n = 55$)	4 (7 %)	51 (93 %)

4 Discussion

Lung cancer is the leading cause of death from cancer in the world. The high mortality rate (85 % within 5 years) results, in part, from the lack of effective tools to diagnose the disease at an early stage. In this study we present the expression level of *FAM107A* in lung tissue obtained from patients with diagnosed non-small cell lung cancer. *FAM107A* is a tumor suppressor gene, localized on 3p, with known decreased expression in several cancer cell lines and few primary cancers. However, regarding lung cancer, it was analyzed only in lung cancer cell lines (Liu et al. 2009; Awakura et al. 2008; Wang et al. 2000). In the present study we confirmed the decreased gene expression in primary lung cancer samples. Similar results were obtained by Liu et al. (2009), who, however, reported decreased or undetectable *FAM107A* expression not on mRNA but on the protein level in 15/20 primary lung cancers and a moderate level in 2/2 normal lung tissues. In the present study, the mean *FAM107A* mRNA expression (RQ value) in macroscopically unchanged lung samples oscillated around 1, the value attributed to the normal lung tissue (calibrator) expression level. The significant differences we found between NSCLC samples and tumor-matched macroscopically unchanged lung tissue specimens could suggest an important role of the *FAM107A* gene in lung tumor development. It might be interesting to examine the expression level of this gene in the patient's blood and to find out if it could serve as a biomarker supporting an early diagnosis of NSCLC. Surrogate tissues, such as bronchial

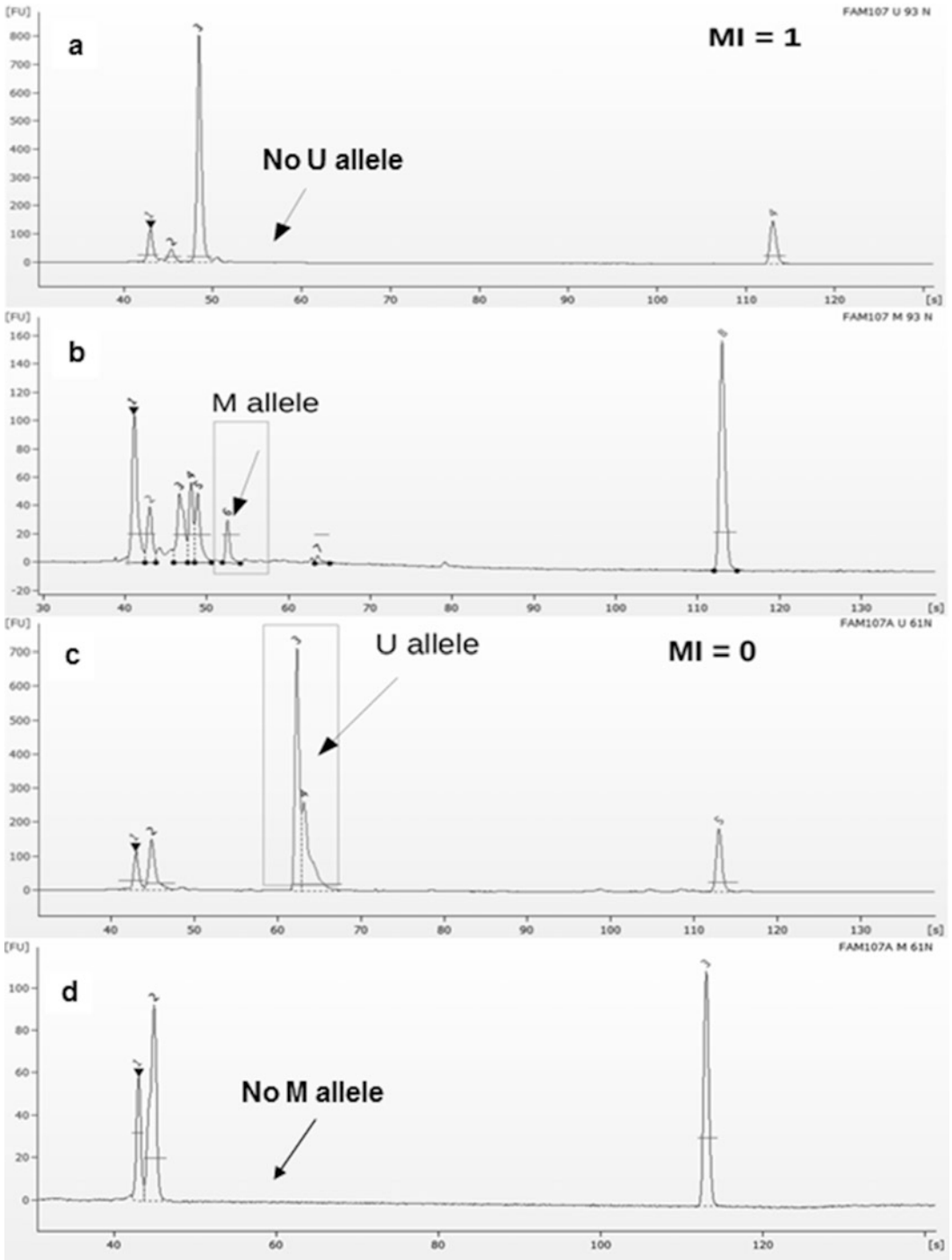


Fig. 3 Examples of methylated (both alleles, MI = 1) and unmethylated (both alleles, MI = 0) NSCLC samples; (a & b) –sample with methylated *FAM107A* gene; (c & d) –sample with unmethylated *FAM107A* gene

brushings and biopsies, as well as biofluids, such as peripheral blood (all its components: circulating cells, plasma, and serum), exhaled breath condensate (EBC), urine, and sputum offer noninvasive methods to obtain large amount of samples for analysis.

In human lung tumor xenografts, *FAM107A* re-expression has been associated with significantly smaller tumor volumes (Liu et al. 2009). In the present study, gene expression was very low and similar regardless of tumor size. It might additionally support the tumor suppressive role of *FAM107A* in lung carcinogenesis.

Smoking is a proven risk factor in lung carcinogenesis (Proctor 2001). Most patients with lung cancer (80–85 %) have a history of smoking, but only a minority of patients who smoke (10–15 %) will actually develop lung cancer (Kathuria et al. 2014). There is no method to precisely predict which current and former smokers will develop lung cancer. Spira et al. (2004) have shown that smoking contributes to the decreased expression of *FAM107A* in the epithelial cells of the pulmonary airway. In our study group, all NSCLC patients, except five persons, were smokers. It is known that the risk of developing lung cancer increases with accumulated exposure to cigarette smoke, and, on the other hand, individuals remain at high risk decades after they have stopped smoking (Ebbert et al. 2003). In the present study we did not find any significant differences between the smoker groups (in relation to PYs) or the NSCLC subtypes while considering only smokers. However, in all those cases *FAM107A* gene expression was dramatically decreased.

Based on the fact that no genetic alterations were found in the *FAM107A* gene (Wang et al. 2000; Yamato et al. 1999), the possibility of the underlying epigenetic mechanism has been taken into consideration. Indeed, *FAM107A* promoter hypermethylation was analyzed in several studies. It has been confirmed in renal cell cancer, bladder cancer, testis cancer, prostate cancer, and astrocytoma (Lin et al. 2013; Awakura et al. 2008; Vanaja et al. 2006; van den Boom et al. 2006). As far as lung cancer is concerned, Awakura et al. (2008) have shown that

methylation of the gene is also present in the lung cancer cell lines. In the present study we did not confirm the pivotal role of promoter methylation of *FAM107A* as the mechanism of gene silencing in our NSCLC patients. However, it should be stressed that epigenetic inactivation of *FAM107A* may occur despite poor methylation. As shown by Zhao et al. (2005), the presence of di-methylation of lysine 9 on histone H3 (H3me2K9) and binding of methyl-CpG binding protein (MeCP2) at the promoter region of TSG are common events leading to gene silencing due to silent heterochromatin state, irrespective of DNA hypermethylation. The heterogeneity of epigenetic regulation should be taken into account. Apart from gene promoter methylation, several other epigenetic mechanisms control gene expression, including post translational modifications to core histones, chromatin remodeling machinery, microRNA (miRNA), and long non-coding RNA (lncRNA) regulation (Gibney and Nolan 2010).

The results obtained in the present study, indicating loss of *FAM107A* expression in NSCLC samples, highlight its importance in lung carcinogenesis. However, it appears that the key *FAM107A* silencing mechanism is not related to the promoter hypermethylation.

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Conflicts of Interest Authors declare no conflicts of interest in relation to this article.

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