Complement factor H polymorphism rs1061170 and the effect of cigarette smoking on the risk of lung cancer

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Aim of the study: Complement factor H (CFH) has been known to inhibit the complement pathway and to contribute to tumour growth by suppressing the anti-tumour cell mediated response in cell lines from several malignancies. We examined the association of Try402His single nucleotide polymorphism in *CFH* gene with lung cancer and the interaction with cigarette smoking.

Material and methods: This case-control study included 80 primary lung cancer patients and 106 control subjects who were genotyped for Try402His (rs1061170) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Results: Variant genotypes (Tyr/His and His/His) were overpresented among patients compared to controls (p = 0.03, OR = 2.510, 95% CI: 1.068–5.899), and the frequency of variant H allele was significantly overexpressed in cases compared to controls (p = 0.021). Tyr/His genotype was identified in 100% of small cell lung cancer (SCLC) patients vs. 34.5% of non-SCLC (NSCLC), while 20.7% of NSCLC patients were homozygous for the variant allele (His/His) (p = 0.001). Binary logistic regression analysis revealed a 2.5 times greater estimated risk for NSCLC than for SCLC among variant allele carriers, and a 7.3-fold increased risk of lung cancer among variant allele smoking carriers vs. 1.3-fold increased risk among wild allele smoking carriers. Moreover, the stage of cancer positively correlated with smoking and pack-years in allele H carriers, and the correlation was stronger among those who were homozygous for it (His/His) than those who were heterozygous (Tyr/His).

Conclusions: CFH 402H variant is a smoking-related risk factor for lung cancer, particularly the NSCLC.

Key words: complement factor H, lung cancer, polymorphism, smoking.

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Introduction

Lung cancer is the leading cause of cancer deaths throughout the world [1]. The five-year survival rate for lung cancer is 15% in developed countries and 5% in many developing countries. These poor survival rates demand new strategies for early detection and major improvements in therapy [2].

New anticancer treatments based on monoclonal antibodies (moAbs) targeted to tumour-associated antigens have recently been proposed. These moAbs can initiate complement-dependent cell lysis [3]. Cancer cells develop mechanisms to avoid immune recognition or activation [4]. So, elucidation of these mechanisms may improve cancer immunotherapy.

The failure of the complement to destroy tumour cells can be partially attributed to their resistance to complement-mediated lysis [5]. This resistance might result from various mechanisms, including the expression of membrane complement regulatory proteins (mCRPs) [6], which normally protect host cells from complement-mediated destruction, and the secretion of soluble complement inhibitors by tumour cells [7].

CD35 (complement receptor type-1 – CR-1), CD46 (membrane cofactor protein – MCP), and CD55 (decay accelerating factor – DAF) are mCRPs that control the activation of complement at the level of C3, which is a central molecule of the complement cascade. Whereas CD59 (membrane inhibitor of reactive lysis – MIRL) interferes with the assembly of the terminal complement complexes [8]. Soluble complement inhibitors, such as C1 inhibitor, factor H, factor-H-like proteins, factor I, and C4b binding protein (C4BP) are secreted by tumour cells into the local microenvironment [9, 10]. C1 inhibitor binds to and inactivates the C1r and C1s proteinases [11], which contribute to the activation of complement through the classical pathway. Factor H is a cofactor for factor-I-mediated cleavage of C3b and accelerates the decay of the alternative pathway C3 convertase [12]. C4BP acts as a cofactor for factor I in the degradation of C3b and C4b. Previous studies demonstrated that various cancer cells express at least one of the mCRPs [5, 13–18]. Because the mechanisms through which moAbs kill tumour cells include antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [19], the presence of mCRPs on cancer cells might impair the therapeutic efficacy of these moAbs [20]. Overcoming the inhibition of complement activation on tumour cells may represent a promising approach for improving the effectiveness of moAbs in the treatment of cancer.

Expression of factor H has been described in primary tumours and cell lines from different origins [21, 22]. Undetectable or low expression of CFH has been identified in breast cancer, prostate cancer, and colon can-

cer cell lines [9]. A single nucleotide polymorphism (SNP), Tyr402His, located in exon 9 of the CFH gene and representing a tyrosine to Histidine change at amino acid position 402 in the CFH protein [23] that alters the complement activity [24], has been reported to be associated with lung cancer [25] and a marker for lung adenocarcinoma [26]. However, its impact on cancer risk is still unclear.

The aim of this work is to investigate CFH Tyr402His SNP in lung cancer patients in a case-control study and to assess its effect with cigarette smoking on the risk of lung cancer in Egyptians.

Material and methods

Study subjects

This case-control study included 80 lung cancer patients who were primary histopathologically confirmed cases previously untreated by radiotherapy and/or chemotherapy, and 106 apparently healthy genetically unrelated subjects with no prior history of malignancy, as controls. Exclusion criteria were patients with previous malignancy or metastatic cancer from other organs.

Full medical history was registered through a questionnaire; thorough clinical examination and chest radiography were performed. All subjects gave written informed consent. The study was approved by the ethical committee of the National Research Centre.

CFH genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells using a QIAamp DNA extraction kit (Qiagen Hilden, Germany, Cat no. 51304) according to the manufacturer's protocol. Genotyping of CFH Tyr402His polymorphism (rs1061170) was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP) analysis as previously described [25]. Briefly, a 244-bp DNA fragment containing the variant site was amplified with the primer pairs of CFH-F (5′- ACT GTG GTC TGC GCT TTT G-3′) and CFR-R (5′- TTT TTG GAT GTT TAT GCA ATC TT-3′). PCR was performed in a 10-µL reaction mixture containing 25 ng DNA, 0.1 mM each primer, and 1 µ Maxima® HotStart Green PCR MasterMix (Thermo Scientific). The thermal profile consisted of an initial denaturation step of 2 minutes at 94°C, followed by 34 cycles of 30 seconds at 94°C, 40 seconds at 60°C, 55 seconds at 72°C, and a final elongation step of 5 minutes at 72°C. PCR product was digested by FastDigest® NlaIII restriction enzyme (Thermo Scientific) at 37°C for 5 minutes. The restriction products were separated on 2% agarose gel and visualised by UV illumination. The 402 Tyr/Tyr genotype had a single 244-bp band; the 402 His/His genotype had two bands, 161-bp and 83-bp, whereas the 402 Tyr/His heterozygous genotype had all three bands: 244-bp, 161-bp, and 83-bp (Fig. 1). A 10% random sample was tested in duplicate and all results were 100% concordant.

Statistical analysis

Data were analysed using SPSS version 16.0 (Chicago, IL, USA). Data were expressed as mean ±SD for continuous variables, or as percentages of total for categorical variables. The Chi Square test was used to compare the distribution of CFH genotypes between the groups. The associations between genotype and risk of lung cancer were estimated by odds ratio (OR) and 95% confidence interval (95% CI) using logistic regression models. The ORs were adjusted for age, smoking status, and pack-years. The Hardy-Weinberg equation was used to calculate the minor allelic frequencies for CFH in cases and controls. Estimated risk and correlations were calculated using regression analysis. *P* value less than 0.05 was considered significant.

Results

A significant age difference was found between controls (mean 49.63 ±6.4 years) and patients (mean 58.38 ±7.5 years) ($p < 0.001$), denoting that older age is associated with higher risk of lung cancer. The frequency of smokers among lung cancer patients was significantly higher than among controls ($p < 0.001$). Mean pack-years was significantly higher in patients (35.3 ±29) than in controls (11.4 ±11.6) ($p = 0.03$). Demographic and clinical characteristics of patients and controls are summarized in Table 1.

Using the Hardy-Weinberg equation, genotyping analysis results showed that the minor allelic frequencies for

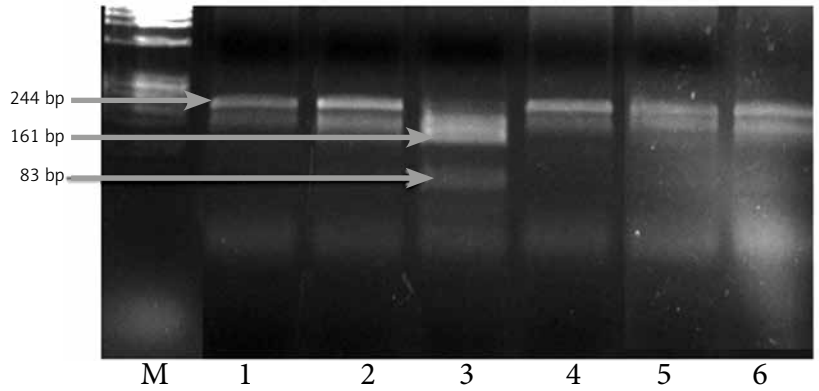

Fig. 1. PCR- RFLP analysis: An agarose gel electrophoresis (2%) illustrating restricted fragments of CFH Tyr402His polymorphism (rs1061170), 244 bp indicates Tyr/Tyr genotype (1,4), 161+83 bp indicates His /His genotype (3), 244+161+83 bp indicates Tyr/His genotype (2,5,6)

Table 1. Demographic and clinical characteristics of patients and controls

Characteristics	Patients (n = 80)		Controls (n = 106)		*P*
	N	(%)	*N*	(%)	
Sex					
Male	64	(80.0)	64	(60.4)	0.07*
Female	16	(20.0)	42	(39.6)	
Age					
Less than 50	16	(20.0)	60	(56.6)	0.04**
51–60	34	(42.5)	46	(43.4)	
More than 60	30	(37.5)	0	(0.0)	
Smoking status					
No	24	(30.0)	56	(52.8)	0.035*
Yes	56	(70.0)	50	(47.2)	
Pack-years					
≤ 20	28	(35.0)	92	(86.8)	0.035*
21–40	24	(30.0)	12	(11.3)	
> 40	28	(35.0)	2	(1.9)	
Histopathology				–	
Adenocarcinoma	38	(47.5)			
Small cell	22	(27.5)			
Large cell	10	(12.5)			
Others[a]	10	(12.5)			
Metastasis				–	
No metastasis	30	(37.5)			
Pulmonary nodules and/or plural effusion	26	(32.5)			
Others (bones, liver, brain, LN)	26	(32.5)			
Staging				–	
I	4	(5)			
II	8	(10)			
III	18	(22.5)			
IV	50	(62.5)			

**Fisher exact test*
***Two-sided χ^2 test*
[a] *Others: undifferentiated (n = 4), mucoepidermoid (n = 2), mesothelioma (n = 2), and mucinous (n = 2).*

CFH were 0.293 (p = 0.102) in controls and 0.413 (p = 0.599) in patients. The frequency of the variant allele-including genotypes (Tyr/His, His/His) was significantly overrepresented in lung cancer patients compared with controls (p = 0.03, OR = 2.510, 95% CI: 1.068–5.899) (Table 2). Analysis of CFH Tyr402His genotypes frequency in small cell lung cancer (SCLC) vs. non-SCLC (NSCLC) showed that 100% of SCLC had Tyr/His genotype. Meanwhile, 34.5% of NSCLC had Tyr/His genotype and 20.7% were homozygous for the variant allele (His/His genotype), p = 0.02, OR = = 0.625, 95% CI: 0.428–0.914 (Table 3).

Binary logistic regression revealed an estimated cancer risk 2.61 times greater for smokers than for non-smokers (p = 0.023). Moreover, a 2.5 times greater estimated risk for NSCLC than for SCLC was identified among the variant allele carriers (p = 0.021).

The risk of lung cancer associated with CFH genotypes was examined by stratification for smoking status; a higher risk of cancer associated with variant genotypes (Tyr/His, His/His) was observed among smokers but not among non-smokers. Binary regression analysis revealed a 7.3-fold increased risk of cancer among smokers with variant allele (Tyr/His, His/His genotypes) vs. 1.3-fold increased risk among smokers with wild genotype (Tyr/Tyr).

Correlation studies showed positive correlation between the stage of cancer and smoking and pack years among Tyr/His genotype carriers (r = 0.496, p = 0.022 and r = 0.530, p = 0.013, respectively), stronger correlations were found among His/His genotype carriers (r = 0.845, p = 0.017 and r = 0.914, p = 0.004, respectively). These significant correlations were lacking among wild genotype (Tyr/Tyr) carriers (r = –0.220, p = 0.469 and r = –0.041, p = 0.895, respectively).

Discussion

The complement system, which plays diverse roles in cancer initiation and development, consists of a cascade of functional proteins for cell lysis [27]. Complement factor H (CFH) is one of the key regulators in the alternative complement pathway, which has been known to inhibit the complement pathway by binding to C3b and destroying the C3 convertase [28, 29]. Lung cancer cells may develop a protective mechanism against complement attack by expressing and binding factor H to their cell membranes. Several studies have also suggested the importance of factor H in the protection of other tumour cells against complement activation [21, 22, 29, 30].

Table 2. Genotypes frequency of CFH Tyr402His SNP in patients and controls

Genotype	Controls (*n* = 106) *N* (%)	Patients (*n* = 80) *N* (%)	*p*	OR (95% CI)
Tyr/Tyr	58 (54.7)	26 (32.5)	0.03	2.510 (1.068–5.899)
Tyr/His	34 (32.1)	42 (52.5)		
His/His	14 (13.2)	12 (15.0)		

Data calculated by binary logistic regression and adjusted for age, smoking status, and pack-years

Table 3. Genotypes frequency of CFH Tyr402His SNP in SCLC vs. NSCLC patients

Genotype	SCLC (*n* = 22) *N* (%)	NSCLC (*n* = 58) *N* (%)	*p*	OR (95% CI)
Tyr/Tyr	–	26 (44.8)	0.02	0.625 (0.428–0.914)
Tyr/His	22 (100)	20 (34.5)		
His/His	–	12 (20.7)		

Data calculated by binary logistic regression and adjusted for age, smoking status, and pack-years

Lung cancer is strongly associated with cigarette smoking, and about 90% of lung cancers arising as a result of tobacco use [31]. Our results showed an estimated risk for lung cancer 2.61 times greater in Egyptian smokers than in non-smokers.

In this study, we demonstrated that CFH Y402H polymorphism is associated with lung cancer. This CFH 402H variant has a remarkably reduced affinity towards C-reactive protein (CRP), which has been implicated to modulate the complement activity via CFH binding [32, 33]. This leads to aberrant regulation of the alternative complement cascade response, excessive inflammation, and tissue damage because of MAC (membrane attack complex) formation [34, 35]. Consecutively, tumour cells could escape the elimination by anti-tumour CD8+ T-cell-mediated response [35]. In a previous study by Zhang *et al.* [25], the frequencies of CFH Y402H genotypes among lung cancer patients were significantly different from those among controls in a Chinese population, with 402His/His or 402His/Tyr genotypes being over-expressed among patients compared with controls (13.6% vs. 9.4%, $p < 0.004$).

Our results showed that CFH 402H carriers have a 2.5-fold increased risk for NSCLC. In a recent study, CFH mRNA expression was demonstrated in 6 out of 10 NSCLC cell lines, but not in SCLC cell lines, and in 54 out of 101 primary lung tumour samples, and higher expression levels significantly correlated with lung adenocarcinoma. Also, survival analysis showed that CFH-positive tumours had worse prognosis compared to CFH-negative tumours. Additionally, a shorter survival time of patients with adenocarcinoma (less than 20 months) was associated with higher CFH protein expression. They concluded that NSCLC cells express and secrete CFH, which might be a novel diagnostic marker for human lung adenocarcinoma [26].

Our results also revealed a 7.3-fold increased risk of lung cancer among variant CFH402H allele smoking carriers and a 1.3-fold increased risk among wild CFH402T allele smoking carriers. Moreover, the stage of cancer positively correlated with smoking and pack-years in 402H carriers, and the correlation was stronger in those who were homozygous for the variant allele (His/His) than in those who were heterozygous for it (Tyr/His), suggesting that CFH Try402His polymorphism is smoking-related risk factor for lung cancer and indicating a strong gene-environment interaction. Zhang *et al.* examined the risk of lung cancer associated with CFH genotypes by stratification for smoking status. A 2.89-fold increased risk was found among smokers with the His-allele-containing genotype, but not among non-smokers [25]. Smoking is the most modifiable environmental risk factor in lung cancer by variant mechanisms; one of these mechanisms is complement activation. The capacity of cigarette smoke to activate the complement system was evaluated and it was found that exposure of serum to cigarette smoke resulted in cleavage of C3 and the generation of C5a [36], which is an important component for alternative complement activity. Kew *et al.* reported that smoke-treated C3 apparently did not bind CFH as C3 or C3b did. They also declared that poor binding of CFH to smoke-treated C3b would theoretically facilitate the activity of alternative complement [37]. This could explain why cigarette smoking modifies the risk of lung cancer in association with CFH genetic variant.

In conclusion, the CFH 402H variant is a risk factor for lung cancer, particularly the NSCLC. Cigarette smoking has a relevant risk-modifying effect, confirming the important role of gene-environment interaction in the development of lung cancer.

The authors declare no conflict of interest.

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