ASSOCIATION BETWEEN THE UROKINASE PLASMINOGEN ACTIVATION SYSTEM POLYMORPHISMS AND NSCLC IN CHINESE POPULATION

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ABSTRACT

Aim*: To evaluate the influence of urokinase plasminogen activation (uPA) and uPAR gene polymorphisms on susceptibility to non-small-cell lung carcinoma (NSCLC).*

Methods: *A total of 500 NSCLC patients and 500 healthy controls were recruited and matched for age and gender. The SNPs distributed in the uPA and uPAR gene were selected for genotyping. The association between genotype and NSCLC risk was evaluated by computing the odds ratio (OR) and 95% confience interval (CI) using multivariate unconditional logistic regression analyses.*

Results: *Patients with the uPAR rs344781 T allele had a reduced risk of developing squamous-cell carcinomas (SCCs) (OR=0.742; 95%CI=0.579–0.950; P=0.0176), especially male patients (OR=0.722; 95%CI=0.546–0.954; P=0.0219). In addition, the uPAR rs344781 C/C allele homozygote was associated with an increased risk of SCC in patients (OR=1.713; 95%CI=1.145–2.563; P=0.0083). However, neither allele frequencies nor genotype frequencies in uPA rs4065 were associated with NSCLC (P>0.05).*

Conclusions: *The results revealed that the genetic polymorphisms of uPAR rs344781 were associated with SCC susceptibility.*

Keywords: *Chinese population, non-small cell lung cancer, uPA, uPAR, polymorphism.*

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Introduction

Extracellular matrix (ECM) degradation mediated by the urokinase plasminogen activation (uPA) system is an important mechanism involved in both physiological and pathological tissue remodeling[1-2]. Many experimental and clinical studies have demonstrated the association of uPA system activity with cancer invasion and metastasis, including lung cancer[3-4]. Members of the uPA system, including uPA and its receptor (uPAR), play critical roles in proteolysis, migration, invasion, and metastasis[5-6].

The median levels of uPA and uPAR expression are higher in lung tumor tissues than the adjacent lung parenchyma. Lung cancer is the leading cause of cancer death worldwide, due to its high mortality and morbidity[7]. In China alone, the incidence and mortality associated with lung cancer are estimated to total 0.7 and 0.6 million cases, respectively[8]. Pathologically, genetic and environmental interactions play a key role in the development and progression of lung cancer[9].

However, these interactions are dependent on genetic variations or single nucleotide polymorphisms (SNPs). Recently, a study in Taiwan showed that genetic polymorphisms of the uPA rs4065 C/T and uPAR rs344781 (-516 T/C) were associated with the susceptibility and severity of non-small-cell

lung carcinoma[NSCLC; 10]. People living in Taiwan and Mainland China have a lot in common, but they have different living environments and diets. In this study, the relationship between the SNPs of uPA, uPAR and NSCLC risk in the Chinese mainland were investigated, and the impact of these SNPs on the susceptibility and clinicopathological characteristics of NSCLC were also evaluated.

Materials and methods

Study population

This study recruited 500 NSCLC patients and 500 unrelated age-matched healthy controls from The Zhejiang Cancer Hospital, Hangzhou, China during the period from March, 2011 to April, 2012. All cases and controls were of Chinese Han origin and lived in Zhejiang Province, China.

Participants had no history of previous primary cancer other than lung cancer. The controls were independent lung-related diseases to avoid probable interferences from overlapping genes. Current smokers or former smokers or non-smokers were included. All subjects provided their informed consent, approved by the Ethic Committee of Zhejiang Cancer Hospital.

SNPs selection and genotyping

uPA rs4065 and uPAR rs344781 were selected according to a previous study[11]. DNA was extracted from whole blood by AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA).

The uPA SNP and uPAR SNP of interest were then genotyped using the SEQUENOM MassARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (Sequenom, San Diego, CA). PCR primers and single base extension primers were designed using Assay Designer's software version 3.0 (Sequenom) and synthesised by Sangon Biotech (Shanghai, China).

The sequences of forward and reverse primers were as follows: 5'-ACGTTGGATGAAGAGACTGGGAAGATAGGC-3' and 5'-ACGTTGGATGGCCTGAGGGTAAAGCTATTG-3'for uPA rs4065 (104 bps); 5'-ACGTTGGATGCACATTCCTTTAACATTTACC-3' and 5'-ACGTTGGATGAACACTTAACCCTTGCTTT-3' for uPAR rs344781 (115 bps).

Statistical analysis

All statistical analyses were performed by using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). The Hardy-Weinberg equilibrium (HWE) was carried out for all SNPs, cases and controls were compared by using the χ^2 test and a P-value of <0.001 was considered statistically different. The χ^2 test was used to assess the frequencies of the selected allele and genotype between the cases and controls.

The association between SNPs and NSCLC risk was analysed by computing the odds ratio (OR) and 95% confidence interval (CI) from multivariate unconditional logistic regression analysis.

A two-sided P<0.05 was considered statistically significant.

Results

500 patients (350 males and 150 females) and 500 healthy controls (259 males and 240 females; gender information for one control subject was missed) of Han origin were tested in this study. For NSCLC patients, 331 had adenocarcinoma (ADC), and 169 had squamous-cell carcinomas (SCC); 280 male patients and 21 female patients were smokers or former smokers, whereas 189 male and 14 female controls were smokers or former smokers in controls. The studied population were within the HWE (P=0.17109 for uPA rs4065; P=0.14444 for uPAR rs344781, respectively).

The allele frequency of uPA rs4065 was 12.6% (T) and 87.4% (C) in NSCLC patients, 12.7% (T) and 87.3% (C) in ADC patients, 12.4% (T) and 87.6% (C) in SCC patients, and 12.5% (T) and 87.5% (C) in controls. No statistical differences in allele frequencies of these four SNPs were found between the case and control subjects (P> 0.05). Stratification by gender revealed no significant difference in allele frequencies (P>0.05). (Table 1, 2, 3).

The allele frequency of uPAR rs344781 was 51.8% (T) and 48.2% (C) in NSCLC patients, 54.2% (T) and 45.8% (C) in ADC patients, 47.0% (T) and 53.0% (C) in SCC patients, and 54.5% (T) and 45.5% (C) in controls. In patients with SCC, the allele frequencies in uPAR rs344781 were significantly different from the controls (OR=0.742; 95%CI=0.579-0.950; P=0.0176).

We then stratified by analysis of gender; the allele frequencies in uPAR rs344781 was significantly different between male SCC patients and male controls (OR=0.722; 95%CI=0.546-0.954; P=0.0219). We found the T allele to be associated with a lower risk for SCC development, and it may be a potential lower-risk marker for male SCC patients in China. (Table 1, 2, 3).

Gene Allele	NSCLC N=500(%)	Controls N=500(%)	*P value*	OR (95%CI)	M NSCLC N=350(%)	M Controls N=259(%)	*P* value	OR (95%CI)	F NSCLC N=150(%)	F Controls N=240(%)	*P* value	OR (95%CI)
uPA rs4065												
T	126 (12.6)	125 (12.5)			86 (12.3)	63 (12.2)			40(13.3)	62 (12.9)		
C	874 (87.4)	875 (87.5)	0.9462	1.009 (0.775–1.315)	614 (87.7)	455 (87.8)	0.9481	1.012 (0.715–1.431)	260 (86.7)	418 (87.1)	0.8667	1.037 (0.677–1.589)
uPAR rs344781												
T	518 (51.8)	545 (54.5)			359 (51.3)	285 (55.0)			159 (53.0)	258 (53.7)		
C	482 (48.2)	455 (45.5)	0.2263	0.897 (0.753-1.070)	341 (48.7)	233 (45.0)	0.1969	0.861 (0.685–1.081)	141 (47.0)	222 (46.3)	0.8381	0.97 (0.727–1.296)

Table 1: Allele frequency of uPA and uPAR SNPs in NSCLC patients and healthy controls.
NSCLC, Non-small cell lung cancer; M, Male; F, Female.

Gene Allele	ADC N=331(%)	Controls N=500(%)	*P* value	OR (95%CI)	M ADC N=189(%)	M Controls N=259(%)	*P* value	OR (95%CI)	F ADC N=142(%)	F Controls N=240(%)	*P* value	OR (95%CI)
uPA rs4065												
T	84 (12.7)	125 (12.5)			47 (12.4)	63 (12.2)			37 (13.0)	62 (12.9)		
C	578 (87.3)	875 (87.5)	0.9095	1.017 (0.757–1.367)	331 (87.6)	455 (87.8)	0.9026	1.026 (0.685–1.535)	247 (87.0)	418 (87.1)	0.9646	1.01 (0.653–1.563)
uPAR rs344781												
T	359 (54.2)	545 (54.5)			208 (55.0)	285 (55.0)			151 (53.2)	258 (53.7)		
C	303 (45.8)	455 (45.5)	0.9137	0.989 (0.812–1.205)	170 (45.0)	233 (45.0)	0.9983	1(0.766-1.306)	133 (46.8)	222 (46.3)	0.8763	0.977 (0.728–1.311)

Table 2: Allele frequency of uPA and uPAR SNPs in ADC patients and controls.
ADC, adenocarcinoma; M, Male; F, Female.

Gene Allele	SCC N=169(%)	Controls N=500(%)	*P* value	OR (95%CI)	M SCC N=161(%)	M Controls N=259(%)	*P* value	OR (95%CI)	F SCC N=8(%)	F Controls N=240(%)	*P* value	OR (95%CI)
uPA rs4065												
T	42 (12.4)	125 (12.5)			39 (12.1)	63 (12.2)			3 (18.8)	62 (12.9)		
C	296 (87.6)	875 (87.5)	0.9716	0.993 (0.684–1.443)	283 (87.9)	455 (87.8)	0.9827	0.995 (0.650–1.524)	13 (81.3)	418 (87.1)	0.4964	1.556 (0.431–5.615)
uPAR rs344781												
T	159 (47.0)	545 (54.5)			151 (46.9)	285 (55.0)			8 (50.0)	258 (53.7)		
C	179 (53.0)	455 (45.5)	0.0176*	0.742 (0.579–0.950)	171 (53.1)	233 (45.0)	0.0219*	0.722 (0.546–0.954)	8 (50.0)	222 (46.3)	0.7673	0.86 (0.318–2.330)

Table 3: Allele frequency of uPA and uPAR SNPs in SCC patients and controls.
*SCC, squamous cell carcinoma; M, Male; F, Female.; *P<0.05.*

Genotype frequencies of uPA rs4065 genotype were 76.2% (C/C), 1.4% (T/T) and 22.4% (C/T) in NSCLC patients, 76.1% (C/C), 1.5% (T/T) and 22.4% (C/T) in ADC patients, 76.3% (C/C), 1.2% (T/T) and 22.5% (C/T) in SCC patients, and 75.8% (C/C), 0.8% (T/T), and 23.4% (C/T) in the controls. No statistical differences in the allele frequencies of these four SNPs were found between the case and control subjects (P>0.05).

Stratification by gender revealed no significant difference in genotype frequencies (P>0.05). Genotype frequencies of uPAR rs344781 were 22.8% (C/C), 26.4% (T/T) and 50.8% (C/T) in NSCLC patients, 19.9% (C/C), 28.4% (T/T) and 51.7% (C/T) in ADC patients, 28.4% (C/C), 22.5% (T/T) and 49.1% (C/T) in SCC patients, and 18.8% (C/C), 27.8% (T/T) and 53.4% (C/T) in the controls. There were statistically significant differences for the C/C, T/T and C/T genotypes between SCC patients and controls (P=0.0263).

When analysing the association between genotypes and the risk of SCC, logistic regression analysis revealed that the uPAR rs344781 C/C allele homozygote was associated with an increased risk of SCC in patients (OR=1.713; 95% CI=1.145-2.563; P=0.0083). (Table 4, 5, 6).

Gene Allele	NSCLC N=500(%)	Controls N=500(%)	*P* value	OR (95%CI)	M NSCLC N=350(%)	M Controls N=259(%)	*P* value	OR (95%CI)	F NSCLC N=150(%)	F Controls N=240(%)	*P* value	OR (95%CI)
uPA rs4065												
C/C	381 (76.2)	379 (75.8)			269 (76.9)	198 (76.4)			112 (74.7)	180 (75.0)		
T/T	7 (1.4)	4 (0.8)			5 (1.4)	2 (0.8)			2 (1.3)	2 (0.8)		
C/T	112 (22.4)	117 (23.4)	0.6273		76 (21.7)	59 (22.8)	0.7271		36 (24.0)	58 (24.2)	0.8925	
T/T+C/T	119 (23.8)	121 (24.2)	0.8823	1.022 (0.765–1.366)	81 (23.1)	61 (23.6)	0.906	1.023 (0.700–1.495)	38 (25.3)	60 (25.0)	0.9411	0.982 (0.614–1.572)
C/C+C/T	493 (98.6)	496 (99.2)	0.3631	1.761 (0.512–6.053)	345 (98.6)	257 (99.2)	0.4525	1.862 (0.358–9.676)	148 (98.7)	238 (99.2)	0.6335	1.608 (0.224–11.539)
uPAR rs344781												
C/C	114 (22.8)	94 (18.8)			85 (24.3)	53 (20.5)			29(19.3)	41 (17.1)		
T/T	132 (26.4)	139 (27.8)			94 (26.9)	79 (30.5)			38(25.3)	59 (24.6)		
C/T	254 (50.8)	267 (53.4)	0.297		171 (48.9)	127 (49.0)	0.4368		83(55.3)	140 (58.3)	0.8079	
TT+CT	386 (77.2)	406 (81.2)	0.1192	1.276 (0.939–1.733)	265 (75.7)	206 (79.5)	0.2653	1.247 (0.845–1.838)	121(80.7)	199 (82.9)	0.5732	1.163 (0.687–1.969)
CC+CT	368 (73.6)	361 (72.2)	0.6185	0.932 (0.705–1.231)	256 (73.1)	180 (69.5)	0.3241	0.837 (0.587–1.193)	112(74.7)	181 (75.4)	0.8676	1.041 (0.650–1.667)

Table 4: Genotypes of uPA and uPAR SNPs in NSCLC patients and controls.
NSCLC, Non-small cell lung cancer; M, Male; F, Female.

Gene allele	ADC N=331(%)	Controls N=500(%)	*P* value	OR (95%CI)	M ADC N=189(%)	M Controls N=259(%)	*P* value	OR (95%CI)	F ADC N=142(%)	F Controls N=240(%)	*P* value	OR (95%CI)
uPA rs4065												
C/C	252 (76.1)	379 (75.8)			145 (76.7)	198 (76.4)			107 (75.4)	180 (75.0)		
T/T	5 (1.5)	4 (0.8)			3 (1.6)	2 (0.8)			2 (1.4)	2 (0.8)		
C/T	74 (22.4)	117 (23.4)	0.5988		41 (21.7)	59 (22.8)	0.7014		33 (23.2)	58 (24.2)	0.8541	
T/T+C/T	79 (23.9)	121 (24.2)	0.9125	1.018 (0.736–1.410)	44 (23.3)	61 (23.6)	0.9466	1.015 (0.652–1.581)	35 (24.6)	60 (25.0)	0.9387	1.019 (0.630–1.648)
C/C+C/T	326 (98.5)	496 (99.2)	0.3326	1.902 (0.507–7.135)	186 (98.4)	257 (99.2)	0.4173	2.073 (0.343–12.528)	140 (98.6)	238 (99.2)	0.5936	1.7 (0.237–12.203)
uPAR rs344781												
C/C	66 (19.9)	94 (18.8)			39 (20.6)	53 (20.5)			27 (19.0)	41 (17.1)		
T/T	94 (28.4)	139 (27.8)			58 (30.7)	79 (30.5)			36 (25.4)	59 (24.6)		
C/T	171 (51.7)	267 (53.4)	0.872		92 (48.7)	127 (49.0)	0.9972		79 (55.6)	140 (58.3)	0.8515	
T/T+C/T	265 (80.1)	406 (81.2)	0.6834	1.076 (0.757–1.528)	150 (79.4)	206 (79.5)	0.9646	1.011 (0.635–1.607)	115 (81.0)	199 (82.9)	0.6335	1.14 (0.666–1.950)
C/C+C/T	237 (71.6)	361 (72.2)	0.8508	1.03 (0.756–1.403)	131 (69.3)	180 (69.5)	0.9664	1.009 (0.672–1.515)	106 (74.6)	181 (75.4)	0.8666	1.042 (0.645–1.682)

Table 5: Genotypes of uPA and uPAR SNPs in ADC patients and controls.
ADC, adenocarcinoma; M, Male; F, Female.

Gene allele	SCC N=169(%)	Controls N=500(%)	*P* value	OR (95%CI)	M SCC N=161(%)	M Controls N=259(%)	*P* value	OR (95%CI)	F SCC N=8(%)	F Controls N=240(%)	*P* value	OR (95%CI)
uPA rs4065												
C/C	129 (76.3)	379 (75.8)			124 (77.0)	198 (76.4)			5 (62.5)	180 (75.0)		
T/T	2 (1.2)	4 (0.8)			2 (1.2)	2 (0.8)			0 (0.0)	2 (0.8)		
C/T	38 (22.5)	117 (23.4)	0.8792		35 (21.7)	59 (22.8)	0.8682		3 (37.5)	58 (24.2)	0.6742	
T/T+C/T	40 (23.7)	121 (24.2)	0.8889	1.03 (0.684–1.551)	37 (23.0)	61 (23.6)	0.893	1.032 (0.648–1.645)	3 (37.5)	60 (25.0)	0.4243	0.556 (0.129–2.394)
C/C+C/T	167 (98.8)	496 (99.2)	0.6476	1.485 (0.270–8.182)	159 (98.8)	257 (99.2)	0.6297	1.616 (0.225–11.590)	8 (100.0)	238 (99.2)	0.7954	
uPAR rs344781												
C/C	48 (28.4)	94 (18.8)			46 (28.6)	53 (20.5)			2 (25.0)	41 (17.1)		
T/T	38 (22.5)	139 (27.8)			36 (22.4)	79 (30.5)			2 (25.0)	59 (24.6)		
C/T	83 (49.1)	267 (53.4)	0.0263*		79 (49.1)	127 (49.0)	0.0753		4 (50.0)	140 (58.3)	0.8299	
T/T+C/T	121 (71.6)	406 (81.2)	0.0083**	1.713 (1.145–2.563)	115 (71.4)	206 (79.5)	0.057	1.555 (0.985–2.453)	6 (75.0)	199 (82.9)	0.5607	1.6189 (0.315–8.301)
C/C+C/T	131 (77.5)	361 (72.2)	0.1757	0.753 (0.500–1.136)	125 (77.6)	180 (69.5)	0.0689	0.656 (0.416–1.035)	6 (75.0)	181 (75.4)	0.9785	1.023 (0.201–5.204)

Table 6: Genotypes of uPA and uPAR SNPs in SCC patients and controls.
*SCC, squamous cell carcinoma; M, Male; F, Female.; *P<0.05; **P<0.01.*

Discussion

In the current study, the association between SNPs (uPA rs4065 and uPAR rs344781) and the risk of developing NSCLC was examined. The results indicate that patients with the uPAR rs344781 T allele had a reduced risk of developing SCC patients, especially a reduced risk of developing male SCC patients. In addition, the uPAR rs344781 C/C allele homozygote was associated with an increased risk of SCC in patients. However, neither allele frequencies nor genotype frequencies in uPA rs4065 were associated with NSCLC.

Tumor invasion and metastasis require proteolytic enzymes that destroy the extracellular matrix and enhance the formation of novel blood vessels[11]. The uPA system is thought to play a role in several different processes which are important to tumor progression, including tissue remodeling, chemotaxis, tumor invasion, dissemination, proliferation and angiogenesis[12-13]. The binding of uPA to uPAR increases the efficiency of plasminogen activation and also serves to localise these proteolytic cascades to the migrating or invading edge of cells[5, 14-15].

The binding of uPA and vitronectin also promotes cell adhesion and cell migration. In addition, uPAR also interacts with various cell surface receptors such as integrins, growth factor receptors and endocytic receptor 180[16-18]. These interactions activate diverse signaling pathways including FAK, Src, MAPK and PI3K, leading to EMT, cell proliferation, cell migration and the expression of pro-cancer genes[5, 19-20]. These signaling pathways play important roles in NSCLC[21-24]. In accordance with these studies, the results of this study indiate that uPAR could be a susceptible gene involved in NSCLC development. In this study, the uPAR rs344781 polymorphism was inversely associated with the development of lung SCC for the Chinese population. C/C homozygote individuals had a 1.713-fold increased risk of developing NSCLC compared with T/T homozygote or C/T heterozygote individuals. In addition, the T allele had a 0.742-fold reduced risk of developing in SCC patients, and a 0.722-fold reduced risk of developing in male SCC patients.

Lung cancer is a complex disease resulting from environmental factors, genetic factors and their interactions[25-26]. The possibility that the relationship between the uPA/uPAR genotypes and NSCLC susceptibility in the study is ethnic-dependent is an observation which cannot be entirely excluded because of the multiple risk factors and etiology contributing to the pathophysiology of NSCLC development. These results are not in agreement with those of Chuen-Ming Shi (10). Together with earlier studies[27-29], these differences may primarily be attributed to the different living environments.

In conclusion, these results reveal that genetic polymorphisms of uPAR rs344781 were associated with the susceptibility of SCC. The results of this study indicate the significant relationship between genetic polymorphisms of uPAR with the susceptibility of SCC. However, the small sample size may be a limitation of the present study. In addition, population stratification may have led to a bias.

References

1) Conway JRW, Jacquemet G. Cell matrix adhesion in cell migration. Essays Biochem 2019; 63: 535-551.
2) Schoen FJ. Morphology, Clinicopathologic Correlations, and Mechanisms in Heart Valve Health and Disease. Cardiovasc Eng Technol 2018; 9: 126-140.
3) Yepes M. Urokinase-type plasminogen activator is a modulator of synaptic plasticity in the central nervous system: implications for neurorepair in the ischemic brain. Neural Regen Res 2020; 15: 620-624.
4) Minopoli M, Botti G, Gigantino V, Ragone C, Sarno S, et al. Targeting the Formyl Peptide Receptor type 1 to prevent the adhesion of ovarian cancer cells onto mesothelium and subsequent invasion. J Exp Clin Cancer Res 2019; 38: 459.
5) Gilder AS, Natali L, Van Dyk DM, Zalfa C, Banki MA, et al. The Urokinase Receptor Induces a Mesenchymal Gene Expression Signature in Glioblastoma Cells and Promotes Tumor Cell Survival in Neurospheres. Sci Rep 2018; 8: 2982.
6) Kristensen G, Berg KD, Lippert S, Christensen IJ, Brasso K, et al. Plasma levels of intact and cleaved urokinase plasminogen activator receptor (uPAR) in men with clinically localised prostate cancer. J Clin Pathol 2017; 70: 1063-1068.
7) Edwards BK, Noone AM, Mariotto AB, Simard EP, Boscoe FP, et al. Annual Report to the Nation on the status of cancer, 1975-2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. Cancer 2014; 120: 1290-1314.
8) Chang ZL, Yang SJ. Analysis of the incidence of breast cancer in Dandong City from 2004 to 2008. Chin J Prevent Control Chronic Dis 2014; 22: 379.
9) Moosazadeh M, Asemi Z, Lankarani KB, Tabrizi R, Maharlouei N, Naghibzadeh-Tahami A, et al. Family history of diabetes and the risk of gestational diabetes mellitus in Iran: A systematic review and meta-analysis. Diabetes Metab Syndr 2017; 11: 99-104.

10) Garona J, Alonso DF. Urokinase Exerts Antimetastatic Effects by Dissociating Clusters of Circulating Tumor Cells-Letter. Cancer Res 2016; 76: 4908.
11) Tsai JP, Hsiao PC, Yang SF, Hsieh SC, Bau DT, et al. Licochalcone A suppresses migration and invasion of human hepatocellular carcinoma cells through downregulation of MKK4/JNK via NF-κB mediated urokinase plasminogen activator expression. PLoS One 2014; 9: 86537.
12) Li H, Chen C. Quercetin Has Antimetastatic Effects on Gastric Cancer Cells via the Interruption of uPA/uPAR Function by Modulating NF-κb, PKC-δ, ERK1/2, and AMPKα. Integr Cancer Ther 2018; 17: 511-523.
13) Wach S, Brandl M, Borchardt H, Weigelt K, Lukat S, et al. Exploring the MIR143-UPAR Axis for the Inhibition of Human Prostate Cancer Cells In Vitro and In Vivo. Mol Ther Nucleic Acids 2019; 16: 272-283.
14) Wang D, Yang Y, Jiang L, Wang Y, Li J, et al. Suppression of Tumor Growth and Metastases by Targeted Intervention in Urokinase Activity with Cyclic Peptides. J Med Chem 2019; 62: 2172-2183.
15) De Lorenzi V, Sarra Ferraris GM, Madsen JB, Lupia M, Andreasen PA, et al. Urokinase links plasminogen activation and cell adhesion by cleavage of the RGD motif in vitronectin. EMBO Rep 2016; 17: 982-998.
16) Liu D, Zhou D, Wang B, Knabe WE, Meroueh SO. A new class of orthosteric uPAR·uPA small-molecule antagonists are allosteric inhibitors of the uPAR·vitronectin interaction. ACS Chem Biol 2015; 10: 1521-1534.
17) Yuan C, Jürgensen HJ, Engelholm LH, Li R, Liu M, et al. Crystal structures of the ligand-binding region of uPARAP: effect of calcium ion binding. Biochem J 2016; 473: 2359-2368.
18) Huang C, Hays FA, Tomasek JJ, Benyajati S, Zhang XA. Tetraspanin CD82 interaction with cholesterol promotes extracellular vesicle-mediated release of ezrin to inhibit tumour cell movement. J Extracell Vesicles 2019; 9: 1692417.
19) Merino P, Diaz A, Torre ER, Yepes M. Urokinase-type plasminogen activator (uPA) regulates the expression and function of growth-associated protein 43 (GAP-43) in the synapse. J Biol Chem 2020; 295: 619-630.
20) Domschke P, Trucu D, Gerisch A, Chaplain MAJ. Structured models of cell migration incorporating molecular binding processes. J Math Biol 2017; 75: 1517-1561.
21) Kitano H, Chung JY, Ylaya K, Conway C, Takikita M, et al. Profiling of phospho-AKT, phospho-mTOR, phospho-MAPK and EGFR in non-small cell lung cancer. J Histochem Cytochem 2014; 62: 335-346.
22) Wang H, Qin M, Liu R, Ding X, Chen ISY, et al. Characterization of A Bifunctional Synthetic RNA Aptamer and A Truncated Form for Ability to Inhibit Growth of Non-Small Cell Lung Cancer. Sci Rep 2019; 9: 18836.
23) Lou W, Chen Y, Zhu KY, Deng H, Wu T, et al. Polyphyllin I Overcomes EMT-Associated Resistance to Erlotinib in Lung Cancer Cells via IL-6/STAT3 Pathway Inhibition. Biol Pharm Bull 2017; 40: 1306-1313.
24) Papadimitrakopoulou V. Development of PI3K/AKT/mTOR pathway inhibitors and their application in personalized therapy for non-small-cell lung cancer. J Thorac Oncol 2012; 7: 1315-1326.
25) Hyndman IJ. Review: the Contribution of both Nature and Nurture to Carcinogenesis and Progression in Solid Tumours. Cancer Microenviron 2016; 9: 63-69.
26) Li Y, Xiao X, Han Y, Gorlova O, Qian D, et al. Genome-wide interaction study of smoking behavior and non-small cell lung cancer risk in Caucasian population. Carcinogenesis 2018; 39: 336-346.
27) Thiesen S, Yin P, Jorgensen AL, Zhang JE, Manzo V, et al. TPMT, COMT and ACYP2 genetic variants in paediatric cancer patients with cisplatin-induced ototoxicity. Pharmacogenet Genomics 2017; 27: 213-222.
28) Rasool Bhat GH, Bhat A, Verma S, Sethi I, Shah R, et al. Genetic variant rs10937405 of TP63 and susceptibility to lung cancer risk in north Indian population. J Genet 2019; 98: 59.
29) Karatza AA, Panagiotopoulou O, Gkentzi D, Dimitriou G. Congenital Heart Disease in Asymptomatic Neonates with Extra-Cardiac Malformations and Genetic Disorders. Balkan Med J 2019; 36: 366.

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