Observational Study

Medicine®

OPEN

Impact of *CCL4* gene polymorphisms upon the progression of lung cancer in a Han Chinese cohort

Weiwei Hu, MD[a], Szu-Yu Chien, PhD[b], Pengqing Ying, MD[a], Po-I Liu, MD[c,d], Chen-Ming Su, PhD[e,*], Chih-Hsin Tang, PhD[b,c,f,g,*]

Abstract

Lung cancer is the most common malignancy in China and has a low survival rate amongst Han Chinese. The high mortality is largely attributed to late-stage diagnosis, when treatment is largely ineffective. Identification of genetic variants could potentially assist with earlier diagnosis and thus more effective treatment. Chemokine (C–C motif) ligand 4 (CCL4) plays a critical role as a chemoattractant in tumor development, metastasis and angiogenesis. In this study, we explored three CCL4 single nucleotide polymorphisms (SNPs) (rs1634507, rs1719153, and rs10491121) in 538 patients with lung cancer and 370 healthy, cancer-free controls. Carriers of the GT + TT heterozygote of rs1634507 had a lower risk of lung cancer than wild-type (GG) carriers, while the presence of the AG + GG heterozygote at rs10491121 was associated with a higher risk of lung cancer compared with having the AA genotype. The G/A/G and T/A/A CCL4 haplotypes significantly reduced and increased the risks for lung cancer, respectively. Our study is the first to document correlations between CCL4 polymorphisms and lung cancer development and progression in people of Han Chinese ethnicity.

Abbreviations: AJCC = American Joint Committee on Cancer, AOR = adjusted odds ratio, CCL4 = Chemokine C motif ligand 4, CI = confidence interval, CIs = confidence intervals, HWE = Hardy–Weinberg equilibrium, IASLC = International Association for the Study of Lung Cancer, MIP-1 = inflammatory protein-1, OR = odds ratios, PCR = polymerase chain reaction, SNP = single nucleotide polymorphisms, TNM = tumor node metastasis.

Keywords: CCL4, gene polymorphism, Han Chinese, lung cancer

1. Introduction

As a result of aging populations, increasing air pollution, cigarette smoking, environmental carcinogens, and genetic predisposition, lung cancer is now the most common malignancy worldwide, constituting around 13% of all cancer cases globally.[1–4] In 2012, worldwide age-standardized mortality rates for males and females with lung cancer were 30.0/100,000 and 11.1/100,000, respectively, reflecting poor 5-year relative survival rates.[5] In 2014, 782,000 new lung cancer cases and 626,000 lung cancer deaths occurred in China.[6] The specific genetic risks underlying lung cancer development and progression remain unclear. Genetic variants and epigenetic changes are known to influence the development and progression of non-small cell lung cancer.[7–9] Thus, specific information about genetic aberrations could potentially assist with risk prediction and earlier diagnosis of lung cancer in individual patients.

Chemokine (C–C motif) ligand 4 (CCL4), also referred to as macrophage inflammatory protein-1 (MIP-1β), is a chemoattractant that plays a critical role in immune response, inflammation and tumor development.[10,11] Increasing evidence indicates that CCL4 controls several tumor-related functions such as proliferation, invasion, metastasis, and angiogenesis.[10,12,13] It has been suggested that high CCL4 concentrations predict unfavorable survival in lung adenocarcinoma.[14] Genetic factors and single nucleotide polymorphism (SNP) genotyping are pivotal in investigations into the risk and prognosis of tumorigenesis.[15–17] SNPs in the *CCL4* chemokine gene are known to be associated with breast cancer, oral cancer, and hepatocellular carcinoma,[18–20] while a recent study has reported

Editor: Undurti N. Das.

This work was supported by grants from China's National Natural Science Foundation (No. 81702117) and China Medical University (CMU108-ASIA-09).

Conflict of interest statement: All authors declare that they have no financial or personal relationships with any other people or organizations that could inappropriately influence this work.

Supplemental Digital Content is available for this article.

[a] Department of Thoracic Surgery, Affiliated Dongyang Hospital of Wenzhou Medical University, Dongyang, Zhejiang, China, [b] School of Medicine, China Medical University, Taichung, Taiwan, [c] Graduate Institute of Biomedical Science, China Medical University, Taichung,, [d] Department of Thoracic Surgery, Changhua Christian Hospital, Changhua, Taiwan, [e] Department of Biomedical Sciences Laboratory, Affiliated Dongyang Hospital of Wenzhou Medical University, Dongyang, Zhejiang, China, [f] Chinese Medicine Research Center, China Medical University, [g] Department of Biotechnology, College of Health Science, Asia University, Taichung, Taiwan.

[*] Correspondence: Chih-Hsin Tang, Department of Pharmacology, School of Medicine, China Medical University, Taichung, Taiwan (e-mail: chtang@mail.cmu.edu.tw), Chen-Ming Su, Department of Biomedical Sciences Laboratory, Affiliated Dongyang Hospital of Wenzhou Medical University (e-mail: proof814@gmail.com).

How to cite this article: Hu W, Chien SY, Ying P, Liu PI, Su CM, Tang CH. Impact of CCL4 gene polymorphisms upon the progression of lung cancer in a Han Chinese cohort. Medicine 2020;99:3(e18906).

Received: 12 July 2019 / Received in final form: 31 October 2019 / Accepted: 23 December 2019

http://dx.doi.org/10.1097/MD.0000000000018906

a protective effect against the risk of developing rheumatoid arthritis in individuals carrying the T-containing genotype of the *CCL4* rs1719153 polymorphism.[21] Thus, the evidence suggests that it is worth seeking associations between *CCL4* gene polymorphisms and lung cancer diagnosis. Our case–control study therefore sought to determine possible associations between three SNPs (rs1634507, rs1719153, and rs10491121) in the *CCL4* gene and lung cancer.

2. Materials and methods

2.1. Patients and blood samples

We collected blood specimens from 538 patients (cases) diagnosed with lung cancer at Dongyang People's Hospital between 2014 and 2018. All patients with lung cancer were diagnosed and were undergoing primary surgical treatment at Affiliated Dongyang Hospital of Wenzhou Medical University (Dongyang, Zhejiang, China). We included normal participants from physical examination center of Dongyang People's Hospital. A total of 370 healthy participants without any history of cancer served as controls. All participants provided written informed consent, and this study has used consecutive or convenient enrollment of subjects. A total of 370 healthy participants without any history of cancer served as controls. All participants provided written informed consent. The study was approved by the Ethics Committee of Dongyang People's Hospital Ethics Committee and Institutional Review Board (2016-YB003). Medical records were reviewed for clinicopathological characteristics. Detailed clinical data on age, sex, smoking history, and alcohol consumption were obtained at baseline from the patients' electronic medical records and all participants completed a standardized questionnaire about demographics and their medical history. Pathological staging data were determined for all patients by their medical records and the Revised International System for Staging Lung Cancer according to the proposed eighth edition of the International Association for the Study of Lung Cancer (IASLC) Tumor Node Metastasis (TNM) Classification.[22] Pathological staging data were determined for all patients by their medical records and the Revised International System for Staging Lung Cancer. Whole blood samples (3 mL) were collected from all study participants and stored at −80°C for subsequent DNA extraction.

2.2. Selection of CCL4 polymorphisms

All three *CCL4* SNPs (rs1634507, rs1719153, and rs10491121) selected for this study had minor allele frequencies of greater than 5%.

2.3. Genomic DNA extraction and genotyping by real-time PCR

Total genomic DNA was isolated from peripheral blood leukocytes using a QIAamp DNA blood mini kit (Qiagen, CA), according to the manufacturer's instructions.[23–25] DNA was dissolved in TE buffer (10 mM Tris, pH 7.8, 1 mM EDTA) and stored at −20°C. All *CCL4* SNP probes were purchased from Thermo Fisher Scientific Inc. (California, CA), and allelic discrimination analysis of *CCL4* SNPs was assessed using a ABI StepOne Real-Time PCR System (Applied Biosystems, CA), according to the manufacturer's instructions.[26,27]

2.4. Statistical analysis

Differences between the two groups were considered to be statistically significant if P values were $<.05$. Hardy–Weinberg equilibrium was assessed using Chi-square goodness-of-fit tests for bi-allelic markers. As the data were independent and normally distributed, Fisher's exact test was used to compare differences in demographic characteristics between healthy controls and patients with lung cancer. As the data were independent and normally distributed, Fisher's exact test was used to compare differences in demographic characteristics between healthy controls and patients with lung cancer. Odds ratios (ORs) combined with 95% confidence intervals (CIs) calculated associations between genotype frequencies and the risk of lung cancer. To further exclude the impact of confounding variables, adjusted odds ratios (AORs) with 95% CIs were estimated by multiple logistic regression models that controlled for confounding covariates. A P value of $<.05$ was considered statistically significant. Data were analyzed using SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC).

3. Results

We enrolled 538 patients with lung cancer and 370 healthy, cancer-free subjects; all were of Han Chinese ethnicity. Between-group differences in general demographic characteristics are listed in Table 1. The mean age was 60.20 ± 10.91 years for the lung cancer cohort and 42.23 ± 19.11 years for the controls ($P < .001$). Significantly higher proportions of lung cancer patients compared with controls were either current or ex-tobacco smokers, or consumed alcohol (both $P < .001$). As according to the American Joint Committee on Cancer (AJCC) tumor/node/metastasis (TNM) classification and staging system,

Table 1

Demographic characteristics of the study population.

Variable	Controls (n = 370) n (%)	Patients (n = 538) n (%)	*P* value
Age, y	42.23 ± 19.11	60.20 ± 10.91	<.001*
Gender			
Male	161 (43.5%)	274 (50.9%)	<.001#
Female	209 (56.5%)	264 (49.1%)	
Alcohol consumption			
No	326 (88.1%)	396 (73.6%)	<.001#
Yes	44 (11.9%)	142 (26.4%)	
Cigarette smoking			
No	325 (87.8%)	335 (62.3%)	<.001#
Yes	45 (12.2%)	203 (37.7%)	
Tumor size (T)			
≤T2		478 (88.8%)	
>T2		60 (11.2%)	
Lymph node status (N)			
N0/N1		437 (81.2%)	
N2/N3		101 (18.8%)	
Distant metastasis (M)			
M0		466 (86.6%)	
M1		72 (13.4%)	
Clinical stage			
I/II		418 (77.7%)	
III/IV		120 (22.3%)	

All values are given as the mean ± SD (standard deviation).

* $p < .05$ versus controls, by Mann–Whitney test.

# $p < .05$ versus controls, by Pearson's Chi-squared test.

Table 2

Genotypic frequencies of the CCL4 rs1634507, rs1719153, and rs10491121 SNPs in cases and controls and the associations between these SNPs and the risk of lung cancer.

Variable	Controls (n = 370) n (%)	Patients (n = 538) n (%)	OR (95% CI)	*P* value	AOR (95% CI)	*P* value
rs1634507						
GG	94 (25.4%)	213 (39.6%)	1.000 (reference)			
GT	175 (47.3%)	242 (45.0%)	0.610 (0.447–0.833)	.002*	0.608 (0.417–0.888)	.010#
TT	101 (27.3%)	83 (15.4%)	0.363 (0.248–0.529)	<.001*	0.395 (0.254–0.616)	<.001#
GT+TT	276 (74.6%)	325 (60.4%)	0.520 (0.388–0.695)	<.001*	0.537 (0.378–0.762)	<.001#
rs1719153						
AA	161 (43.5%)	228 (42.4%)	1.000 (reference)			
AT	164 (44.3%)	240 (44.6%)	1.033 (0.779–1.372)	.820	1.032 (0.729–1.461)	.857
TT	45 (12.2%)	70 (13.0%)	1.098 (0.718–1.681)	.665	1.090 (0.658–1.807)	.738
AT+TT	209 (56.5%)	310 (57.6%)	1.047 (0.802–1.368)	.734	1.045 (0.754–1.449)	.790
rs10491121						
AA	160 (43.2%)	133 (24.7%)	1.000 (reference)			
AG	165 (44.6%)	272 (50.6%)	1.983 (1.468–2.678)	<.001*	1.994 (1.383–2.876)	<.001#
GG	45 (12.2%)	133 (24.7%)	3.556 (2.362–5.351)	<.001*	3.348 (2.072–5.411)	<.001#
AG+GG	210 (56.8%)	405 (75.3%)	2.320 (1.746–3.082)	<.001*	2.317 (1.640–3.275)	<.001#

All values are the given as the mean ± SD (standard deviation). The odds ratios (ORs) with their associated 95% confidence intervals (CIs) were estimated by Pearson's Chi-squared test. The adjusted odds ratios (AORs) with their 95% CIs were estimated by multiple logistic regression modeling that controlled for age, gender, alcohol consumption and cigarette smoking.

* $p < .05$ versus controls, by Pearson's chi-squared test.

\# $p < .05$ versus controls, by multiple logistic regression analyses.

we identified 418 patients (77.7%) with clinical stage I/II and 120 patients (22.3%) with clinical stage III/IV disease (Table 1). To reduce the possible interference of confounding variables, AORs with 95% CIs were estimated by multiple logistic regression models after controlling for age, gender, alcohol, and tobacco consumption in each comparison.

The results of genotyping for the three *CCL4* SNPs (rs1634507, rs1719153, and rs10491121) in the cases and controls are shown in Table 2. In both study groups, the alleles with the highest distribution frequency for rs1634507, rs1719153, and rs10491121 were heterozygous GT, heterozygous AT, and heterozygous AG, respectively (Table 2). Individuals carrying the GT + TT heterozygote at rs1634507 had a 0.537-fold lower risk of lung cancer compared with individuals carrying the wild-type GG polymorphic allele (95% CI: 0.378–0.762, $P < .001$), while those carrying the AG + GG heterozygote at rs10491121 had a 2.317-fold higher risk of lung cancer compared with individuals carrying the wild-type AA polymorphic allele (95% CI: 1.640–3.275, $P < .001$) (Table 2).

Next, *CCL4* genotypes in patients with lung cancer were evaluated to clarify the role of *CCL4* polymorphisms in TNM stage, primary tumor size, lymph node metastasis and distant metastasis. In all 538 patients, no significant differences were observed between *CCL4* rs10491121 genotypes and clinicopathological status (Table 3; the data are not shown for rs1634507 and rs1719153).

Table 3

Odds ratios (ORs) and their associated 95% confidence intervals (CIs) for clinical status and CCL4 rs10491121 genotypic frequencies in patients with lung cancer.

rs10491121	Patients (n = 538) n (%)		OR (95% CI)	*P* value	AOR (95% CI)	*P* value
	Clinical stage					
	I/II	III/IV				
AA	110 (20.4%)	23 (4.3%)	1.000 (reference)			
AG	205 (38.1%)	67 (12.5%)	1.563 (0.923–2.648)	.095	1.308 (0.751–2.277)	.344
GG	103 (19.1%)	30 (5.6%)	1.393 (0.760–2.554)	.283	1.245 (0.647–2.397)	.512
AG+GG	308 (57.2%)	97 (18.0%)	1.506 (0.910–2.493)	.110	1.275 (0.750–2.168)	.369
	Tumor size (T)					
	≤T2	≤T2				
AA	122 (22.7%)	11 (2.0%)	1.000 (reference)			
AG	239 (44.4%)	33 (6.1%)	1.531 (0.748–3.135)	.241	1.195 (0.558–2.558)	.646
GG	117 (21.7%)	16 (3.0%)	1.517 (0.676–3.404)	.310	1.297 (0.541–3.112)	.560
AG+GG	356 (66.2%)	49 (9.1%)	1.527 (0.769–3.030)	.224	1.224 (0.593–2.526)	.585
	Lymph node status (N)					
	N0/N1	N0/N1				
AA	113 (21.0%)	20 (3.7%)	1.000 (reference)			
AG	215 (40.0%)	57 (10.6%)	1.498 (0.857–2.617)	.154	1.260 (0.706–2.251)	.434
GG	109 (20.3%)	24 (4.5%)	1.244 (0.650–2.381)	.509	1.059 (0.528–2.124)	.872
AG+GG	324 (60.2%)	81 (15.1%)	1.412 (0.828–2.410)	.204	1.192 (0.683–2.080)	.536
	Distant metastasis (M)					
	M0	M0				
AA	118 (21.9%)	15 (2.8%)	1.000 (reference)			
AG	230 (42.8%)	42 (7.8%)	1.437 (0.765–2.697)	.258	1.251 (0.654–2.393)	.499
GG	118 (21.9%)	15 (2.8%)	1.000 (0.4.68–2.138)	1.000	0.955 (0.432–2.108)	.908
AG+GG	348 (64.7%)	57 (10.6%)	1.289 (0.703–2.362)	.411	1.136 (0.609–2.117)	.688

All values are given as the mean ± SD (standard deviation). The ORs with their associated 95% CIs were estimated by Pearson's Chi-squared test. The adjusted odds ratios (AORs) with their 95% CIs were estimated by multiple logistic regression modeling that controlled for age, gender, alcohol consumption, and cigarette smoking.

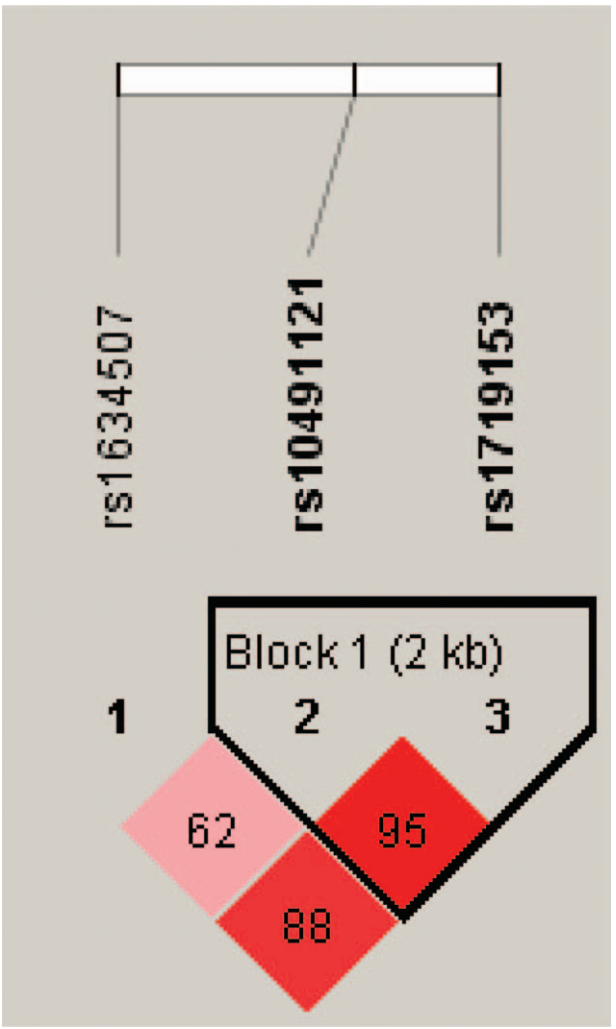

Figure 1. Pairwise linkage disequilibrium (*D'*) patterns of three single nucleotide polymorphisms in the CCL4 gene. CCL4 = Chemokine C motif ligand 4

A reconstructed linkage disequilibrium plot of the genotyped polymorphisms in the study population is depicted in Figure 1. *CCL4* rs10491121 and rs1719153 displayed 95% linkage disequilibrium, while rs1634507 and rs10491121 expressed 88% linkage disequilibrium. The most common haplotype, G/A/G in healthy controls, was used as the reference for analysis distribution frequencies of all 3 polymorphisms examined in this study (rs1634507, rs1719153, and rs10491121). The G/A/G *CCL4* haplotype significantly reduced the risk of developing lung cancer by 0.440-fold (95% CI: 0.016–0.120), while the T/A/A *CCL4* haplotype significantly enhanced the risk by 7.156-fold (95% CI: 4.526–11.315) (Table 4).

4. Discussion

It is recognized that several SNP genes affect an individual's susceptibility to lung cancer.[7,28,29] Correlations between *CCL4* gene polymorphisms and lung cancer have rarely been documented; most of the evidence is associated with breast cancer, oral cancer and hepatocellular carcinoma.[18–20] A comprehensive understanding about genetic polymorphisms is crucial for the successful identification of novel therapeutic strategies for lung cancer.[7,30] To the best of our knowledge, our study provides the first clinical evidence on associations between *CCL4* polymorphisms (rs1634507, rs1719153, and rs10491121) and lung cancer susceptibility, as well as on interactions between these polymorphisms and clinical characteristics in a Han Chinese population. In this study, we increased our overall numbers of participants by incorporating the patients and controls included in our previous study.[8] Our results show that *CCL4* gene polymorphisms only slightly affect the susceptibility for lung cancer. This might be because most of our study participants (cases and controls alike) were nonsmokers and because the cigarette smoker/nonsmoker ratios for both patients (37.7:62.3) and controls (12.2:87.) were relatively normally distributed, as were the proportions of those who consumed alcohol. In this study, we included all the cases and controls without matching age and gender, so that we analyzed the multiple logistic regression modeling statistics by controlled for confound variables. The statistic results between OR and AOR which controls for age and gender were approaching though controls group is younger than cases group, suggesting that CCL4 SNPs were high risks in lung cancer patients. Although our results demonstrated that tobacco smoking and alcohol consumption were significant risk factors for lung cancer (Table 1; both $P < .001$), the significance of this association did not persist in *CCL4* SNPs genotyping analyses that controlled for smoking and alcohol consumption (Supplementary Table S1, http://links.lww.com/MD/D654). These results suggest that smoking and alcohol consumption would not the primary factors for CCL4 SNPs risks in LC patients, and whether there are other factors, such as air pollution or circumstances, it requires to be further considerate in the future.

Table 4

Haplotype frequencies of three CCL4 polymorphisms (rs1634507, rs1719153, and rs10491121) and the risk of lung cancer.

rs1634507	rs1719153	rs10491121	Controls (n = 740) n (%)	Patients (n = 1,076) n (%)	OR (95% CI)	*P* value
G/T	A/T	A/G				
G	A	A	358 (48.4%)	504 (46.8%)	1.000 (reference)	
G	A	G	4 (0.5%)	128 (11.9%)	0.440 (0.016–0.120)	<.001*
T	A	A	122 (16.5%)	24 (2.2%)	7.156 (4.526–11.315)	<.001*
T	T	G	249 (33.6%)	342 (31.8%)	1.025 (0.829–1.267)	.820
Other			7 (0.9%)	78 (7.2%)	0.126 (0.058–0.277)	<.001*

Values are given as the mean ± SD (standard deviation). The odds ratios (ORs) with their associated 95% confidence intervals (CIs) were estimated by the Pearson's Chi-squared test. The "Other" group included G/T/T, G/T/G, T/A/G, and T/T/A haplotypes.

* $p < .05$ versus controls, by multiple logistic regression analysis.

Accumulating evidence indicates that CCL4 plays a critical role in different tumor cell types.[31–33] For example, CCL4 enhances prostate cancer progression via STAT3-dependent signaling.[34] A previous report has suggested that the AT haplotype and T alleles of rs1719153 occurring in *CCL4* SNPs are more frequently expressed in healthy controls than in patients with HIV-1 infection.[35] To the best of our knowledge, this current study is the first to investigate the distribution of the *CCL4* SNPs rs1634507, rs1719153 and rs10491121 and their possible association with susceptibility to lung cancer. In analyses adjusting for confounding factors, the rs1634507 GT + TT heterozygote polymorphism was associated with a significantly lower risk of developing lung cancer compared to the presence of the rs10491121 GG homozygous polymorphism. Moreover, individuals carrying the AG + GG heterozygote at rs10491121 had a higher risk of lung cancer compared with individuals carrying the wild-type AA polymorphic allele.

Linkage disequilibrium can be used for the genetic mapping of adjacent variants that participate in the detection and treatment of disease, while haplotype analyses clarify genetic contribution to disease susceptibility.[36,37] Here, our results indicated that *CCL4* rs10491121 and rs1719153 displayed 95% linkage disequilibrium, whereas rs1634507 and rs10491121 expressed 88% linkage disequilibrium. We also show that the G/A/G *CCL4* haplotype significantly reduced the risk of lung cancer development, while the T/A/A *CCL4* haplotype significantly enhanced the risk, suggesting that these *CCL4* haplotypes play an important role in lung cancer progression.

In summary, our results demonstrate significant associations between *CCL4* SNPs rs1634507 and rs10491121 and the risk of lung cancer in a Han Chinese population. This study is the first to report any such correlation between *CCL4* polymorphisms and lung cancer. Our evidence indicates that *CCL4* could be developed as a genetic prognostic marker for lung cancer prognosis.

Author contributions

Data curation: Weiwei Hu, Pengqing Ying, Chen-Ming Su.
Formal analysis: Weiwei Hu, Szu-Yu Chien, Chen-Ming Su.
Resources: Po-I Liu.
Supervision: Chen-Ming Su, Chih-Hsin Tang.
Writing – original draft: Chih-Hsin Tang.
Writing – review & editing: Chih-Hsin Tang.
Chih-Hsin Tang orcid: 0000-0002-7113-8352.

References

[1] Parra C, Jodar-Sanchez F, Jimenez-Hernandez MD, et al. Development, implementation, and evaluation of a telemedicine service for the treatment of acute stroke patients: TeleStroke. Interact J Med Res 2012;1:e15.
[2] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
[3] Liu JF, Lee CW, Tsai MH, et al. Thrombospondin 2 promotes tumor metastasis by inducing matrix metalloproteinase-13 production in lung cancer cells. Biochem Pharmacol 2018;155:537–46.
[4] Yang YC, Chiou PC, Chen PC, et al. Melatonin reduces lung cancer stemness through inhibiting of PLC, ERK, p38, beta-catenin, and Twist pathways. Environ Toxicol 2019;34:203–9.
[5] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–386.
[6] Chen W, Sun K, Zheng R, et al. Cancer incidence and mortality in China, 2014. Chin J Cancer Res 2018;30:1–2.
[7] Hu W, Liu PY, Yang YC, et al. Association of HMGB1 gene polymorphisms with lung cancer susceptibility and clinical aspects. Int J Med Sci 2017;14:1197–202.
[8] Hu WW, Tang CH, Sun Y, et al. Correlation between resistin gene polymorphism and clinical aspects of lung cancer. Medicine 2017;96: e9485.
[9] Huang CY, Ju DT, Chang CF, et al. A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. Biomedicine 2017;7:23.
[10] Lien MY, Tsai HC, Chang AC, et al. Chemokine CCL4 induces vascular endothelial growth factor C expression and lymphangiogenesis by miR-195-3p in oral squamous cell carcinoma. Front Immunol 2018;9:412.
[11] Mollica Poeta V, Massara M, Capucetti A, et al. Chemokines and chemokine receptors: new targets for cancer immunotherapy. Front Immunol 2019;10:379.
[12] Sivina M, Werner L, Rassenti L, et al. Dynamic changes in CCL3 and CCL4 plasma concentrations in patients with chronic lymphocytic leukaemia managed with observation. Brit J Haematol 2018;180: 597–600.
[13] Wang Y, Liu T, Yang N, et al. Hypoxia and macrophages promote glioblastoma invasion by the CCL4-CCR5 axis. Oncology Rep 2016;36:3522–8.
[14] Li L, Liu YD, Zhan YT, et al. High levels of CCL2 or CCL4 in the tumor microenvironment predict unfavorable survival in lung adenocarcinoma. Thorac Cancer 2018;9:775–84.
[15] Wang B, Yeh CB, Lein MY, et al. Effects of HMGB1 polymorphisms on the susceptibility and progression of hepatocellular carcinoma. Int J Med Sci 2016;13:304–9.
[16] Wang CQ, Tang CH, Wang Y, et al. FSCN1 gene polymorphisms: biomarkers for the development and progression of breast cancer. Sci Rep 2017;7:15887.
[17] Yang MD, Lin KC, Lu MC, et al. Contribution of matrix metalloproteinases-1 genotypes to gastric cancer susceptibility in Taiwan. Biomedicine 2017;7:10.
[18] Hu GN, Tzeng HE, Chen PC, et al. Correlation between CCL4 gene polymorphisms and clinical aspects of breast cancer. Int J Med Sci 2018;15:1179–86.
[19] Lien MY, Lin CW, Tsai HC, et al. Impact of CCL4 gene polymorphisms and environmental factors on oral cancer development and clinical characteristics. Oncotarget 2017;8:31424–34.
[20] Wang B, Chou YE, Lien MY, et al. Impacts of CCL4 gene polymorphisms on hepatocellular carcinoma susceptibility and development. Int J Med Sci 2017;14:880–4.
[21] Kuo SJ, Huang CC, Tsai CH, et al. Chemokine C-C motif ligand 4 gene polymorphisms associated with susceptibility to rheumatoid arthritis. Biomed Res Int 2018;2018:9181647.
[22] Rami-Porta R, Bolejack V, Crowley J, et al. The IASLC Lung Cancer Staging Project: proposals for the revisions of the T descriptors in the forthcoming eighth edition of the TNM classification for lung cancer. J Thorac Oncol 2015;10:990–1003.
[23] Lee HP, Chen PC, Wang SW, et al. Plumbagin suppresses endothelial progenitor cell-related angiogenesis in vitro and in vivo. J Funct Foods 2019;52:537–44.
[24] Liu SC, Tsai CH, Wu TY, et al. Soya-cerebroside reduces IL-1 beta-induced MMP-1 production in chondrocytes and inhibits cartilage degradation: implications for the treatment of osteoarthritis. Food Agr Immunol 2019;30:620–32.
[25] Lee H-P, Wang S-W, Wu Y-C, et al. Glucocerebroside reduces endothelial progenitor cell-induced angiogenesis. Food Agric Immunol 2019;30:1033–45.
[26] Wang L, Tang CH, Lu T, et al. Resistin polymorphisms are associated with rheumatoid arthritis susceptibility in Chinese Han subjects. Medicine 2018;97:e0177.
[27] Wang B, Hsu CJ, Chou CH, et al. Variations in the AURKA gene: biomarkers for the development and progression of hepatocellular carcinoma. Int J Med Sci 2018;15:170–5.
[28] Liu C, Cui H, Gu D, et al. Genetic polymorphisms and lung cancer risk: Evidence from meta-analyses and genome-wide association studies. Lung Cancer 2017;113:18–29.
[29] Huang CY, Hsieh MJ, Wu WJ, et al. Association of endothelial nitric oxide synthase (eNOS) polymorphisms with EGFR-mutated lung adenocarcinoma in Taiwan. J Cancer 2018;9:2518–24.

[30] Ishikawa T. Genetic polymorphism in the NRF2 gene as a prognosis marker for cancer chemotherapy. Front Genet 2014;5:383.
[31] Sasaki S, Baba T, Nishimura T, et al. Essential roles of the interaction between cancer cell-derived chemokine, CCL4, and intra-bone CCR5-expressing fibroblasts in breast cancer bone metastasis. Cancer Lett 2016;378:23–32.
[32] Nguyen-Hoai T, Pham-Duc M, Gries M, et al. CCL4 as an adjuvant for DNA vaccination in a Her2/neu mouse tumor model. Cancer Gene Ther 2016;23:162–7.
[33] Takahashi K, Sivina M, Hoellenriegel J, et al. CCL3 and CCL4 are biomarkers for B cell receptor pathway activation and prognostic serum markers in diffuse large B cell lymphoma. Brit J Haematol 2015;171: 726–35.
[34] Fang LY, Izumi K, Lai KP, et al. Infiltrating macrophages promote prostate tumorigenesis via modulating androgen receptor-mediated CCL4-STAT3 signaling. Cancer Res 2013;73:5633–46.
[35] Modi WS, Lautenberger J, An P, et al. Genetic variation in the CCL18-CCL3-CCL4 chemokine gene cluster influences HIV Type 1 transmission and AIDS disease progression. Am J Hum Genet 2006;79:120–8.
[36] Shifman S, Bronstein M, Sternfeld M, et al. A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 2002;71:1296–302.
[37] Zhang L, Zhang Y, Tang CH, et al. RAD52 gene polymorphisms are associated with risk of colorectal cancer in a Chinese Han population. Medicine 2017;96:e8994.