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Association between variants of *IL-8* and *IL-10* genes, and efficacy of transcatheter arterial chemoembolization and subsequent prognosis in patients with liver cancer

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Abstract. – OBJECTIVE: Our objective was to examine the association between single nucleotide polymorphisms of interleukin (IL)-8 (rs4073 and rs2227306) and IL-10 (rs1800871 and rs1800872) genes, and clinical effects of transcatheter arterial chemoembolization (TACE) and subsequent prognosis in patients with liver cancer.

PATIENTS AND METHODS: 115 patients with liver cancer underwent TACE. Venous blood specimens were collected for genomic DNA extraction. The restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) analysis was used to detect the above variants of *IL-8* and *IL-10* genes. In addition, blood levels of alpha fetal protein (AFP) were quantified by radioimmunoassay. Patients were followed up to uncover the association of the above genotypes with treatment efficacy and survival.

RESULTS: Patients with the homozygous genotype AA or homozygous genotype TT (respectively, -251 and +781 sites) of *IL-8* gene, and wild-type genotype TT or homozygous genotype AA (respectively, -819 and -592 sites) of *IL-10* gene showed the best effectiveness of TACE. Furthermore, these patients also exhibited the lowest AFP levels and the longest survival after the treatment.

CONCLUSIONS: Clinical efficacy of TACE and patient survival in liver cancer are associated with specific variants of *IL-8* and *IL-10* genes.

Key Words:
Interleukin-8, Interleukin-10, Gene polymorphism, TACE, Correlation.

Introduction

Liver cancer is a common malignant cancer of the digestive system. The morbidity caused by liver cancer reaches 624,000 per year[1-3]. The diagnosis is difficult to make at early stages. Therefore, patients are diagnosed with a high malignancy grade cancer, with abundant vascularization, vigorous metabolism, and frequent metastasization. The prognosis is poor and associated mortality is high[4-6].

Interleukin (IL)-8 and IL-10 are chemotactic factors that belong to the CXC gene family. These interleukins modulate chemotaxis and adhesion of neutrophils, and are associated with proliferation and regeneration of tumour vascular endothelial cells and tumour metastasis[7-9]. Overexpression of these interleukins is associated with gross tumour volume, envelope deficiency, vascular invasion, tumour stage, and patient survival time[10-13]. In this study, we tested the association of polymorphisms of *IL-8* and *IL-10* genes, and efficacy of transcatheter arterial chemoembolization (TACE) and post-treatment survival in patients with liver cancer.

Patients and Methods

Patients

One hundred and fifteen patients who were diagnosed with liver cancer and admitted to the Department of Invasive Technology of our Hospital from January 1, 2011, through June 30, 2012, were enrolled in this study. There were 84 male and 31 female patients. The patient age ranged between 31 and 77 years (mean ± SD age of 57.1 ± 6.1 years). Disease course ranged between 2 and 12 (4.1 ± 1.2) weeks. Sixty-one patients had highly differentiated and 54 poorly differentiated cancer.

No patients with surgical contraindication, pregnancy, embryo-derived tumour, active liver disease, psychiatric history or poor compliance

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were also included. The predicted survival time of all patients was more than 6 months.

Treatment

All patients signed informed consent to operation and received TACE. TACE included infusion of 40-60 mg of epirubicin, 100-200 mg of oxaliplatin, and 0.5-1.0 g of fluorouracil, and embolism with 3-25 ml of iodized oil emulsion. All interventions conformed to operation steps of liver cancer surgery. Patients received routine treatment after the surgery. Blood pressure, heart rates, pulse and respiration were monitored. Patients had absolute bed rest within 6 hours after the operation. The puncture point was closed by pressure using a sandbag. The diet and patient activity were guided.

Instruments and Analyses

The instruments included thermocycler (Bio-Rad, Hercules, CA, USA), SW-CJ-1D clean bench (Jiangsu Sujing, Suzhou, China), DK-8D electric heating thermostatic water bath (Shanghai Jinghong Laboratory Equipment, Shanghai, China), DYY-8 electrophoresis apparatus of stable voltage and current (Shanghai Tanon Technology, Shanghai, China), YXJ-2 centrifuge (Jintan Instrument, Shanghai, China), H6-1 miniature electrophoresis tank (Shanghai Tanon Technology), gel imaging system (Shanghai Tanon Technology), U-3010 ultraviolet-visible spectrophotometer and pipettor (Hitachi, Tokyo, Japan), ACS180 automatic chemiluminescence apparatus and the matching kits (Bayer AG, Leverkusen, Germany).

The utilized reagents were DNA extraction kits (Beijing Bio-tech Biological Technology, Beijing, China), and TaqDNA polymerase, PCR product purification kit, and restriction enzymes (all from Roche Diagnostics, Basel, Switzerland). The kit to quantify he tumour marker Alpha Fetal Protein (AFP) was from China Institute of Atomic Energy (Beijing, China).

Genotyping of Variants of IL-8 and IL-10 by RFLP-PCR

To extract DNA, 3 ml of peripheral venous blood were collected from patients and preserved at -20º C. The SNP sites were selected for detection, and potential functional sites with minimum allele frequency of > 0.05 were selected from dbSNP database. The PCR reaction (15 μl) was assembled using 0.5 μl of template DNA, 1.5 1 of 10× Taq buffer, 0.3 μl of 2.5 mM dNTP, 0.2 μl of 50 pmol/μl forward and reverse primers, 1.2 μl of 1.7 mmol/L $MgCl_2$, and 0.5 U of Taq. PCR reaction proceeded after initial denaturation at 95º C (5 min) for 20 cycles of 95º C (30 sec), 68º C (45 sec) and 72º C (60 sec), followed by a single cycle of 95º C (30 sec), 58º C (30 sec) and 72º C (40 sec), and incubation at 72º C for 6 min.

Restriction digestion was done overnight at 37º C in a total volume of 15 μl using 10 μl of PCR reaction and 1 U of appropriate restriction enzymes.

The tested SNP were the following: rs4073, rs2227306, rs1800871, rs1800872. rs4073 was tested using forward primer 5'-GATTGGCTGGCTTATCTTCA-3' and reverse primer 5'-CAAATACGGAGTATGACGAAAG-3'; when digested with restriction enzyme Mun I, PCR product yielded 170 bp and 102 bp fragments with the genotype AA and 272 bp, 170 bp and 102 bp fragments with the genotype AT. rs2227306 was amplified using forward primer 5'-gcggtcccaaaagggtcagtGTGGTATCACAGAGGATTATGC-3' and reverse primer 5'-gcggtccaaaagggtcagtCAGTCATAACTGACAACATTGATC-3'; its restriction enzyme digestion with BclI yielded a 162 bp fragment with the genotype CC, 118 bp and 44 bp fragments with the genotype TT, and 168 bp, 118 bp, and 44 bp fragments with the genotype CT. rs1800871 was tested using forward primer 5'-gcggtccaaaagggtcagtCAAGGTTTCATTCTATGTGCTGG-3' and reverse primer 5'-gcggtccaaaagggtcagtGCAAACTGAGGCACAGGGAT-3', with digestion by FokI yielding 156 bp fragment (TT genotype), 102 bp and 54 bp fragments (CC genotype), or 156 bp, 102 bp, and 54 bp fragments (CT genotype). Finally, rs1800872 was amplified using forward primer 5'-GAGCACTACCTGACTAGCATATAAG-3' and reverse primer 5'-GTGGGCTAAATATCCTCAAAGT-3'; this SNP was digested with RsaI to yield a 244 bp fragment (CC genotype), 173 bp and 71 bp fragments (AA genotypes), or 244 bp, 173 bp, and 71 bp fragments (AC genotypes).

Assessment of TACE Efficacy

Treatment efficacy was assessed according the RECIST 1.1 criteria[2]. Treatment efficacy was ranked as complete response, partial response, stable disease, and progressive disease. The sum of complete and partial response, and stable disease was defined as total effectiveness. Patient distributions in each genotype were observed and

compared with efficacy of TACE (size of the lesion and change of AFP levels). In addition, we compared patient survival with different genotypes at 6 months, 1 year, and 2 years after TACE.

Serum AFP Levels

Radioimmunoassay was utilized to quantify AFP, using acridinium ester as a substrate. AFP levels between 20-400 μg/L were considered as normal.

Statistical Analysis

The data are presented as mean ± SD. Comparisons among groups were done using one-way ANOVA. A $p < 0.05$ was considered as statistically significant.

Results

TACE Efficacy in Patients with Different Genotypes

TACE was most effective, and survival was the longest, in patients with homozygous genotype AA or homozygous genotype TT (respectively, -251 and +781 sites) of *IL-8* gene, and wild-type genotype TT or homozygous genotype AA (respectively, -819 and -592 sites) of *IL-10* gene (Table I).

Association Between AFP levels, and IL-8 and IL-10 genotypes

Patients with *IL-8* genotypes -251AA and +781TT, and *IL-10* genotypes -592AA, -819TT, and -592AA most frequently had normal AFP levels ($p < 0.05$; Table II).

Association Between Survival Time, and IL-8 and IL-10 Genotypes

Patients with *IL-8* genotypes -251AA and -+781TT, and *IL-10* genotypes -592AA, -819TT, and -592AA exhibited the longest survival ($p < 0.05$; Table III).

Discussion

Presently, the assessment of clinical efficacy of TACE mainly relies on imaging techniques[14-17]. The drawback of this approach is that tumour lesions consist of parenchymal tumour cells and connective tissue in patients with primary liver cancer. During TACE, parenchymal tumour cells

Table I. Association between genetic variants of IL-8 and IL-10, and efficacy of TACE.

		CR (n = 17)			PR (n = 26)			SD (n = 41)			PD (n = 31)		
		Number of patients	%	p	Number of patients	%	p	Number of patients	%	p	Number of patients	%	p
IL-8 (-251) (rs4073)	AA	9	52.94	<0.05	13	50.00	<0.05	22	53.66	<0.05	15	48.39	<0.05
	CC	5	29.41		7	26.92		9	21.95		7	22.58	
	TT	3	17.65		6	23.08		10	24.39		9	29.03	
IL-8 (+781) (rs2227306)	AA	2	11.76	<0.05	6	23.08	<0.05	12	29.27	<0.05	10	32.26	<0.05
	CC	5	29.42		5	19.23		10	24.39		5	16.13	
	TT	10	58.82		15	57.69		19	46.34		16	51.61	
IL-10 (-819) (rs1800871)	AA	2	11.76	<0.05	7	26.92	<0.05	13	31.70	<0.05	7	22.59	<0.05
	CC	4	23.54		8	30.77		8	19.52		6	19.35	
	TT	11	64.70		11	42.31		20	48.78		18	58.06	
IL-10 (-592) (rs1800872)	AA	9	52.94	<0.05	13	50.00	<0.05	26	63.41	<0.05	17	54.84	<0.05
	CC	7	41.18		5	19.23		7	17.07		5	16.13	
	TT	1	5.88		8	30.77		8	19.52		9	29.03	

Footnote: Clinical effects were ranked as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to RECIST 1.1 criteria.

Table II. Association between genetic variants of *IL-8* and *IL-10*, and AFP levels.

AFP levels (μg/L)	*IL-8*		*IL-10*		
	-251 (rs4073)	+781 (rs2227306)	-819 (rs1800871)	-592 (rs1800872)	*p*
<20	5 (4.34)	7 (6.09)	9 (7.83)	8 (6.96)	N.S.
20 – 400	47 (40.87)	27 (23.48)	13 (11.30)	51 (44.35)	<0.05
>400	18 (15.65)	11 (9.57)	14 (12.17)	20 (17.39)	<0.05

Footnote: N.S.: not significant.

Table III. Association between genetic variants of *IL-8* and *IL-10*, and survival time.

Survival time	*IL-8*		*IL-10*		
	-251 (rs4073)	+781 (rs2227306)	-819 (rs1800871)	-592 (rs1800872)	*p*
6 months	9 (7.82)	15 (13.04)	11 (9.57)	8 (6.96)	<0.05
1 year	41 (35.65)	22 (19.13)	15 (13.04)	47 (40.87)	<0.05
2 years	10 (8.71)	18 (15.65)	13 (11.30)	21 (18.26)	<0.05

Footnote: N.S.: not significant.

are killed, while connective tissue survives. Therefore, the diagnosis and curative effect assessment by imaging are not sufficiently accurate[18-20].

It was reported that *IL-8* modulates proliferation of endothelial cells in tumours and vessels, as well as regeneration of the tumour and its metastasization[21]. In patients with big tumour lesions at advanced TNM stages, *IL-8* is highly expressed and may serve as a prognostic biomarker[21-23]. Our results demonstrate that the prognosis of patients with homozygous *IL-8* genotypes AA (-251) and TT (+781) is more favourable, which is consistent with previous reports[23]. This indicates that *IL-8* polymorphism can exert some effect on the tumour.

IL-10 is a cytokine secreted by Th2 cells to inhibit Th1 cells. *IL-10* is in the key immunoregulating gene, with dual immunosuppressing and immunostimulating functions. Similar to *IL-8*, expression of *IL-10* is associated with the prognosis in patients with liver cancer[13,24,25]. Specifically, the prognosis of patients with wild-type genotype TT (-819 site) or homozygous genotype AA (-592) was favourable. However, this observation may be confounded by patients' race and environment. In our study, all patients were Chinese and originated from the same province (residents of Nantong area), which helped to maintain a homogenic patient population.

Our results indicate that lower AFP levels are associated with homozygous *IL-8* genotypes AA (-251 site) and TT (+781 site), as well as with wild-type *IL-10* genotype TT (-819 site) and homozygous genotype AA (-592 site). These genotypes also predicted longer survival times. Some studies demonstrate that *IL-8* -251 genetic variant rs4073 is related to capsule invasion and metastasis, and is thus involved in the infiltration of the tumour[26]. The *IL-10* -592 variant rs1800872 modulates activation of the transcription factor Nuclear Factor (NF)-κB and the inflammatory cytokine Tumour Necrosis Factor (TNF)-α[27,28]. TNF-α and NF-κB can stop gene expression and cell proliferation without killing hepatic cells. Many studies demonstrated that TNF-α is closely associated with chronic hepatitis B and liver cancer[29,30].

Conclusions

Our results support the hypothesis that efficacy of TACE and subsequent patient survival are associated with polymorphisms of *IL-8* and *IL-10* genes.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Kawaguchi T, Kohjima M, Ichikawa T, Seike M, Ide Y, Mizuta T, Honda K, Nakao K, Nakamuta M, Sata M. The morbidity and associated risk factors of cancer in chronic liver disease patients with diabetes mellitus: a multicenter field survey. J Gastroenterol 2015; 50: 333-341.
2) Causey MW, Nelson D, Johnson EK, Maykel J, Davis B, Rivadeneira DE, Champagne B, Steele SR. The impact of Model for End-Stage Liver Disease-Na in predicting morbidity and mortality following elective colon cancer surgery irrespective of underlying liver disease. Am J Surg 2014; 207: 520-526.
3) Wolf PS, Park JO, Bao F, Allen PJ, DeMatteo RP, Fong Y, Jarnagin WR, Kingham TP, Gonen M, Kemeny N, Shia J, D'Angelica MI. Preoperative chemotherapy and the risk of hepatotoxicity and morbidity after liver resection for metastatic colorectal cancer: a single institution experience. J Am Coll Surg 2013; 216: 41-49.
4) Chapiro J, Lin M, Duran R, Schernthaner RE, Geschwind JF. Assessing tumor response after loco-regional liver cancer therapies: the role of 3D MRI. Expert Rev Anticancer Ther 2015; 15: 199-205.
5) Pollack HJ, Kwon SC, Wang SH, Wyatt LC, Trinh-Shevrin C. Chronic hepatitis B and liver cancer risks among Asian immigrants in New York City: Results from a large, community-based screening, evaluation, and treatment program. Cancer Epidemiol Biomarkers Prev 2014; 23: 2229-2239.
6) Mishra MN, Vangara KK, Palakurthi S. Transcriptional targeting of human liver carboxylesterase (hCE1m6) and simultaneous expression of anti-BCRP shRNA enhances sensitivity of breast cancer cells to CPT-11. Anticancer Res 2014; 34: 6345-6351.
7) Burgazli KM, Venker CJ, Mericliler M, Atmaca N, Parahuleva M, Erdogan A. Importance of large conductance calcium-activated potassium channels (BKCa) in interleukin-1b-induced adhesion of monocytes to endothelial cells. Eur Rev Med Pharmacol Sci 2014; 18: 646-656.
8) Ozler A, Turgut A, Sak ME, Evsen MS, Soydinc HE, Evliyaoglu O, Gul T. Serum levels of neopterin, tumor necrosis factor-alpha and Interleukin-6 in preeclampsia: relationship with disease severity. Eur Rev Med Pharmacol Sci 2012; 16: 1707-1712.
9) Shelbaya S, Amer H, Seddik S, Allah AA, Sabry IM, Mohamed T, El Mosely M. Study of the role of interleukin-6 and highly sensitive C-reactive protein in diabetic nephropathy in type 1 diabetic patients. Eur Rev Med Pharmacol Sci 2012; 16: 176-182.
10) Singha B, Phyo SA, Gatla HR, Vancurova I. Quantitative analysis of bortezomib-induced IL-8 gene expression in ovarian cancer cells. Methods Mol Biol 2014; 1172: 295-304.
11) Britschgi A, Radimerski T, Bentires-Alj M. Targeting PI3K, HER2 and the IL-8/JAK2 axis in metastatic breast cancer: Which combination makes the whole greater than the sum of its parts? Drug Resist Updat 2013; 16: 68-72.
12) Miteva LD, Stanilov NS, Deliysky TS, Stanilova SA. Significance of -1082A/G polymorphism of IL10 gene for progression of colorectal cancer and IL-10 expression. Tumour Biol 2014; 35: 12655-12664.
13) Franks HA, Wang Q, Lax SJ, Collins MK, Escors D, Patel PM, Jackson AM. Novel function for the p38-MK2 signaling pathway in circulating CD1c+ (BDCA-1+) myeloid dendritic cells from healthy donors and advanced cancer patients; inhibition of p38 enhances IL-12 whilst suppressing IL-10. Int J Cancer 2014; 134: 575-586.
14) Huppert P, Wenzel T, Wietholtz H. Transcatheter arterial chemoembolization (TACE) of colorectal cancer liver metastases by irinotecan-eluting microspheres in a salvage patient population. Cardiovasc Intervent Radiol 2014; 37: 154-164.
15) Martin RC, Robbins K, Tomalty D, O'Hara R, Bosnjakovic P, Padr R, Rocek M, Slauf F, Scupchenko A, Tatum C. Transarterial chemoembolisation (TACE) using irinotecan-loaded beads for the treatment of unresectable metastases to the liver in patients with colorectal cancer: an interim report. World J Surg Oncol 2009; 7: 80.
16) Kuran S, Ozin Y, Nessar G, Turhan N, Sasmaz N. Is endorectal ultrasound still useful for staging rectal cancer? Eur Rev Med Pharmacol Sci 2014; 18: 2857-2862.
17) Ursino S, Fiorica F, Stefanelli A, Pedriali M, Colosimo C, Cocuzza P, Mazzotti V, Taibi R, Cartei F, Greco C. Anaplastic Thyroid Cancer: a case report of a long term survival patient and review of literature data. Eur Rev Med Pharmacol Sci 2014; 18: 1368-1372.
18) Loffroy R, Favelier S, Cherblanc V, Estivalet L. C-arm dual-phase cone-beam CT: a revolutionary real-time imaging modality to assess drug-eluting beads TACE success in liver cancer patients. Quant Imaging Med Surg 2013; 3: 196-199.
19) Santeufemia DA, Lumachi F, Fadda GM, Lo Re G, Miolo G, Basso SM, Chiara GB, Tumolo S. Comment on "repetitive transarterial chemoembolization (TACE) of liver metastases from gastric cancer: local control and survival results": will there be clinical implications in the future? Eur J Radiol 2013; 82: 1591-1592.
20) Vogl TJ, Gruber-Rouh T, Eichler K, Nour-Eldin NE, Trojan J, Zangos S, Naguib NN. Repetitive transarterial chemoembolization (TACE) of liver metastases from gastric cancer: local control and survival results. Eur J Radiol 2013; 82: 258-263.

21) Hartman ZC, Poage GM, den Hollander P, Tsimelzon A, Hill J, Panupinthu N, Zhang Y, Mazumdar A, Hilsenbeck SG, Mills GB, Brown PH. Growth of triple-negative breast cancer cells relies upon coordinate autocrine expression of the proinflammatory cytokines IL-6 and IL-8. Cancer Res 2013; 73: 3470-3480.

22) Hanker LC, Rody A, Holtrich U, Pusztai L, Ruckhaeberle E, Liedtke C, Ahr A, Heinrich TM, Sanger N, Becker S, Karn T. Prognostic evaluation of the B cell/IL-8 metagene in different intrinsic breast cancer subtypes. Breast Cancer Res Treat 2013; 137: 407-416.

23) Zuccari DA, Leonel C, Castro R, Gelaleti GB, Jardim BV, Moscheta MG, Regiani VR, Ferreira LC, Lopes JR, Neto Dde S, Esteves JL. An immunohistochemical study of interleukin-8 (IL-8) in breast cancer. Acta Histochem 2012; 114: 571-576.

24) Hussain SK, Madeleine MM, Johnson LG, Du Q, Galloway DA, Daling JR, Malkki M, Petersdorf EW, Schwartz SM. Nucleotide variation in IL-10 and IL-12 and their receptors and cervical and vulvar cancer risk: a hybrid case-parent triad and case-control study. Int J Cancer 2013; 133: 201-213.

25) Razmkhah M, Jaberipour M, Erfani N, Habibagahi M, Talei AR, Ghaderi A. Adipose derived stem cells (ASCs) isolated from breast cancer tissue express IL-4, IL-10 and TGF-beta1 and upregulate expression of regulatory molecules on T cells: do they protect breast cancer cells from the immune response? Cell Immunol 2011; 266: 116-122.

26) Ahirwar DK, Mandhani A, Mittal RD. IL-8 -251 T > A polymorphism is associated with bladder cancer susceptibility and outcome after BCG immunotherapy in a northern Indian cohort. Arch Med Res 2010; 41: 97-103.

27) Lampiasi N, Azzolina A, D'Alessandro N, Umezawa K, McCubrey JA, Montalto G, Cervello M. Antitumor effects of dehydroxymethylepoxyquinomicin, a novel nuclear factor-kappaB inhibitor, in human liver cancer cells are mediated through a reactive oxygen species-dependent mechanism. Mol Pharmacol 2009; 76: 290-300.

28) Kee JY, Ito A, Hojo S, Hashimoto I, Igarashi Y, Tsuneyama K, Tsukada K, Irimura T, Shibahara N, Takasaki I, Inujima A, Nakayama T, Yoshie O, Sakurai H, Saiki I, Koizumi K. CXCL16 suppresses liver metastasis of colorectal cancer by promoting TNF-alpha-induced apoptosis by tumor-associated macrophages. BMC Cancer 2014; 14: 949.

29) Hsu YL, Kuo PL, Cho CY, Ni WC, Tzeng TF, Ng LT, Kuo YH, Lin CC. Antrodia cinnamomea fruiting bodies extract suppresses the invasive potential of human liver cancer cell line PLC/PRF/5 through inhibition of nuclear factor kappaB pathway. Food Chem Toxicol 2007; 45: 1249-1257.

30) Cesaratto L, Codarin E, Vascotto C, Leonardi A, Kelley MR, Tiribelli C, Tell G. Specific inhibition of the redox activity of ape1/ref-1 by e3330 blocks tnf-alpha-induced activation of IL-8 production in liver cancer cell lines. PLoS One 2013; 8: e70909.