LEADMAN 检验视界

2015 2

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SA

床 义：意临

唾液酸测定试剂盒用于体外定量测定人体血清或血浆中唾液酸的含量。人体中唾液酸主要来源于葡萄糖代谢的中间产物，其值在发生炎症性疾病及恶性肿瘤时升高，例如肺癌、胃癌、肠癌、肝癌、卵巢癌等。因此，测定血清中的唾液酸是诊断这些疾病、观察治疗过程及诊断愈后判定的重要指标；亦可作为普通人群肿瘤筛查的生物标记物。

滕大志

A PROMISE FOR A BETTER LIFE

势 德 SA 定 测 剂盒曼 试利 檫量 优

- 利德曼采用神经氨酸苷酶法，灵敏度高。试剂盒在高、中、低浓度样本检测中均表现出良好的精密度；
- 试剂盒抗干扰能力强，若待测血清无严重的溶血、乳糜和黄疸均可以正常检测；
- 长达 18 个月的有效期以及半个月的定标周期，稳定的试剂状态保证结果的可靠，方便与科室的日常操作；
- 试剂为液体双试剂，比例为 3:1，利德曼配有多种类型试剂瓶规格，适用于市面上绝大多数全自动生化分析仪，上机即用，使用方便；
- 原料到成品全程质量控制，真正保证试剂品质。

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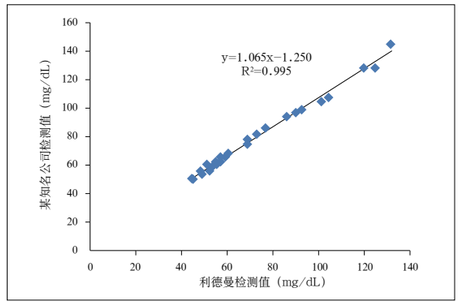

< 利德曼 SA 与某知名品牌试剂相关性 >

Times	15	15	15
Concentration	35.73 mg/dL	67.42 mg/dL	95.27 mg/dL
SD	0.37	0.56	1.01
CV	1.03%	0.84%	1.06%

< 利德曼 SA 的低、中、高浓度测值精密度 >

订货信　　息

产品名称	唾液酸测定试剂盒—SA（神经氨酸苷酶法）
包装规格	R1:2x60mL R2:2x20mL
	质控品（选配） 2x1mL
	校准品（选配） 1x1mL

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CONTENTS

2020

B X X

(CT) (MRI) SPECT

(PET)

AFP AFU

AFP AFU

AFP AFU PHC

98.85%

PG

PGI PGI/II

(Circulating Tumor Cells, CTCs)

CTCs

CA125 CA153 CA549[6-10]

PSA

[1]

1846

Bence-Jones

B-J

B-J

1928 1963

B. Zondek C. Markert

1.

“ ”

1963 1969

G. I. Abelev P. Gold and S. Freeman[2-4]

α- AFP

(CEA)

1975

1.

	AFP	70k	
	CEF	600k	
	CEA	22k	
	POA	40k	
	SCC	44-48k	
	TPA	45k	

2.

carbohydrate antigen

CA

2.

	125	CA125	200k	
	15-3	CA15-3	400k	
	549	CA549		
	27.29	CA27.29		
	DU-PAN-2	DU-PAN-2	100-500k	
	CA19-9	CA19-9	Lexa	
	19-5	CA19-5	Le^a Le^{ag}	
	CA50	CA50	Le^a	
	CA72-4	CA72-4	Tn	
	CA242	CA242	CHO	
		SCC	44-48k	

3.

3.

	ALD	160k	
	ALP	95k	
	AMY	45k	
	GST	80k	
	CK	83k	
γ-	GGT	90k	
	LDH	135k	
	NSE	73k	
5-	5-NT	70K	
α-L	AFU	230k	
	RNASE	20K	
	PSA	34k	

4.

4

	ACTH	4.5k	
	ADH		
	CT	3.5k	
	GH	21k	
	hCG	45k	
	HPL	22k	
	PTH	9k	
	PRL	22k	
	GLG	3k	
	TGF	25K	

5.

β2

5.

β2-	β2M	12k	B Waldenstrom ' s
C	C-P	3.6k	
	FER	450k	
	BJP	22.5-45k	
	IgG	160-900k	
	CP	126-160k	
	TG	670k	

6.

[11]

6 +

	CA19-9 CEA CA72-4,ALP	GGT
	NMP22 TPA CEA CA50	APP
	TPA CEA, PTH	B-ALP HYP
	CA15-3 MCA CA549 CEA	SHR Her-2/neu
/	NSE SCC CYF21-1 CEA, PTH,PRL, ADH, ACTH,FER GH CT	APP
/	SCC CEA CA125 TPA HCG,	APP
	HCG β-HCG	
/	CEA CA19-9 TPA FER	APP
	AFP CEA CA19-9 FER	L-Enz
	S-100 FER	MEL
/	CA72-4 CA19-9 CEA SCC FER	GAS
	CA125 CA72-4 CA15-3 CEA	APP
	CA19-9 CEA CA125 TPA FER GLG	INS GAS
	t-PSA,f-PSA,C-PSA,PAP,FER	APP
	CA72-4 CA19-9 CEA TPA FER	GAS
	AFP β-HCG HCG LDH	
/ /	SCC CEA FER	EBV
	TG NSE CEA TPA CT	

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2008 409-42.

AFP

I

5

HRP

(α-fetoprotein AFP) ELISA

AFP 0.5ng/ml

T

1

AFP

AFP

,

AFP 4 CTL T

AFP CTL AFP

3

4~5 1:10

1.1

1.1.1

6 BALB/c

SP2/0

1.1.2 AFP Biospecific HisTrap

HP HiTrap Protein G GE 50%

PEG 1450 HAT HT

HRP IgG TMB

Sigma DMEM Gibco

AFP Biospecific > 90%

1.2

1.2.1 RT-PCR AFP 100 mg RNA RT-PCR 50 30min, 94 2 min 94 45s, 58 30s, 72 45s, 33 , 72 7min PCR cDNA , : 94 2 min, 94 30 s, 61 30 s, 72 30 s 33 , 72 7 min

1.2.2

AFP-1: P1 5'-CATGCCATGGGACATTCAGAC-3'
P2 5'-CCGCTCGAGGCATTCAACTGC-3'
AFP-2: P1 5'-CATGCCATGGAACGTGGTCAATG-3'
P2 5'-CCGCTCGAGCTCCTGGTATCC-3'
AFP-3: P1 5'-CATGCCATGGGACACTTATGTATC-3'
P2 5'-CCGCTCGAGCTCTTGCTTCATCG-3'

AFP-1 AFP-2 AFP-3 RT-PCR A FP P1 P2 DNA 3 DNA pET-28a(+) DH5α 1.2.6 LB Nco Xho TaKaRa

1.2.3 BL21(DE3) LB 37 1 100 LB 37 OD1.0 1mM IPTG 4 HisTrap HRP HP

LB 10g/L 5g/L NaCl 10g/L 121 20min LB 26

1.2.4 6 BALB/c 50μg/ 4 25μg/ 2 25μg/ 1:10W 3 25μg

1.2.5 1×10^8 Sp2/0 1×10^7 % 50% PEG1450 HAT 7d HT 14 d DMEM 20% ELISA 200ng/ml 3~5 100%

1.2.7 4 30min 30 min pH 9.0 4 4 2 h 4 30 min pH7.4 AFP ELISA

1.2.8 ELISA ELISA 0.01mol/L pH9.6 5μg/ml 4 PBS/T20 3 3 min 5 PBS/T20 37 1 h AFP 37 45 min HRP- 37 30 min TMB 15 AFP 37 15 min 450 nm OD

2

2.1

TaKaRa GenBank ,

2.2 AFP

AFP 2 BALB/c 7 96 1~2 100 4~5 7 21

2.3 AFP

2.3.1 AFP ELISA 20 1:6W~1:20W 1:500~1:5K 1:200~2K

2.3.2 ELISA AFP 3 ELISA 3D5 AFP-2 7B11 0.5ng/ml AFP

(Tumor markers TM) 77.1% AFP>500μg/L 18%
AFP
2. AFP
<300μg/L, <100μg/L
AFP
AFP
AFP
3. AFP
4. 3 AFP 7 8
400μg/L

50(CA50)

CA50

CA50

94.4% 88%
(88%) 80%

(AFP)

AFP

AFP

1000~5000μg/L 1
20μg/L 70
AFP 400μg/L
400μg/L

0~20μg/L (3 -6) 0 ~39μg/L

1. AFP

0 ~ 23kU/L

1.
CA50
2.
CA50 CA50
3.
CA50

(SCC)

SCC
TA-4 2.5μg/L SCC

0 ~ 1.5 ng/mL

1. SCC
SCC
2.
SCC

α-L- (AFU)

AFU
AFU 3.

AFP

AFU

(NSE)

NSE
SCLC 91%
NSCLC

NSE

0~20 ng/mL

1. NSE
(NSCLC)

NSE
NSE NSE
4 12
2. NSE Wilms
NSE

(NFFA)

AFU AFP NFFA

0~40U/L

1. AFU AFP
AFU
AFP
AFU 0.1 0.76 mmol/L
2. NAFE
3. AFU AFU
Reye

I

sialic acid SA) N-

NANA ,

SA

SA

SA

1

SA CEA

SA

CEA

2

SA , SA

73.7% 90.1%

86.0% SA CEA 20%

,SA CEA

SA

SA

SA

SA

SA ( )

3

SA

CEA SF

96.1% , SA

4

SA 80.4%

87.3%

SA TSGF

89.8% SA TSGF

SA TSGF

5

SA 70-80%

SA SA

6

(SA)

SA SA

SA

7

SA

SA SA

SA SA

SA

SA

SA

SA

SA

SA CEA AFP CA

125 CA 50

SA

SA

SA

SA

SA

SA

SA

SA SA

SA

SA

SA

SA

,

SA

3:1

18

1. .
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16-18.
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AFP AFU

PHC

AFU [5]

Primary hepatic carcinoma,PHC

PHC

[1]

AFP

PHC AFP

PHC AFU

B CT AFP PHC PHC

AFP AFP AFU PHC

98.85%

PHC [2] PHC

AFP AFP

AFP [3] >400ng/ml >200ng/ml

8 SGPT

AFP

PHC 30%~40%

AFP

AFU α—

AFP>200ng/ml

AFP>500ng/ml >342umol/L

AFP AFU

1980 deugnier[4] AFU

90% AFU AFU

AFU AFP [J]. 2000,15 6 361—362

[3] .AFP AFP 405 [J]. 2002 17 2 87—89

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[1] . AFU [J]. 2004 12 2 97.

[2] .

circulating tumor cells, AKA CTCs

l

hepatocellular carcinoma, AKA HCC EpCAM
HCC) CTCs NMLD
EpCAM CTC CTC DNA
HCC HCC FFPE PBMC
DNA
NMLD

(Hepatocellular carcinoma,
HCC) (Circulating tumor cells,
CTCs) EpCAM

HCC NMLD
CellSearch CTCs
EpCAM

CTCs DNA

20 HCC 10 NMLD
20 HCC 7
CTCs CTCs ≥ 2/7.5 mL (35%, 95%
12% 60%) 9 NMLD p=0.04
CTCs CTCs ≥
1/7.5 mL alpha-fetoprotein ≥
400 ng/mL (p = 0.008) CTC DNA
HCC 100
CTCs 43% PMBC 87%
CTCs p < 0.001)

CellSearch
CellSearch EpCAM
Veridex LLC, Raritan, New Jersey, U.S.A EpCAM
FDA EpCAM
CTC

EpCAM EpCAM
HCC CTCs DNA
CTCs 30%
80% CellSearch CTCs DNA
CTCs
DNA

CTCs HCC
HCC
CellSearch
CellSearch CTCs HCC
HCC EpCAM

EpCAM HCC
CTC
CTC CTC HCC
CTCs CTCs DNA HCC
CTCs “ ” CTC
CTC HCC

CTC DNA
CTC
CTCs
DNA Illumina MiSeq DNA HCC
CTC
CTC
HCC HCC
DNA CTC

EpCAM

Kelley et al. BMC Cancer (2015) 15:206
DOI 10.1186/s12885-015-1195-z

RESEARCH ARTICLE Open Access

Circulating tumor cells in hepatocellular carcinoma: a pilot study of detection, enumeration, and next-generation sequencing in cases and controls

Robin K Kelley[1*], Mark Jesus M Magbanua[2], Timothy M Butler[3], Eric A Collisson[2], Jimmy Hwang[2], Nikoletta Sidiropoulos[4], Kimberley Evason[5], Ryan M McWhirter[2], Bilal Hameed[6], Elizabeth M Wayne[7], Francis Y Yao[8], Alan P Venook[1] and John W Park[2]

Abstract

Background: Circulating biomarkers are urgently needed in hepatocellular carcinoma (HCC). The aims of this study were to determine the feasibility of detecting and isolating circulating tumor cells (CTCs) in HCC patients using enrichment for epithelial cell adhesion molecule (EpCAM) expression, to examine their prognostic value, and to explore CTC-based DNA sequencing in metastatic HCC patients compared to a control cohort with non-malignant liver diseases (NMLD).

Methods: Whole blood was obtained from patients with metastatic HCC or NMLD. CTCs were enumerated by CellSearch then purified by immunomagnetic EpCAM enrichment and fluorescence-activated cell sorting. Targeted ion semiconductor sequencing was performed on whole genome-amplified DNA from CTCs, tumor specimens, and peripheral blood mononuclear cells (PBMC) when available.

Results: Twenty HCC and 10 NMLD patients enrolled. CTCs ≥ 2/7.5 mL were detected in 7/20 (35%, 95% confidence interval: 12%, 60%) HCC and 0/9 eligible NMLD ($p = 0.04$). CTCs ≥ 1/7.5 mL was associated with alpha-fetoprotein ≥ 400 ng/mL ($p = 0.008$) and vascular invasion ($p = 0.009$). Sequencing of CTC DNA identified characteristic HCC mutations. The proportion with ≥ 100x coverage depth was lower in CTCs (43%) than tumor or PBMC (87%) ($p < 0.025$). Low frequency variants were higher in CTCs ($p < 0.001$).

Conclusions: CTCs are detectable by EpCAM enrichment in metastatic HCC, without confounding false positive background from NMLD. CTC detection was associated with poor prognostic factors. Sequencing of CTC DNA identified known HCC mutations but more low-frequency variants and lower coverage depth than FFPE or PBMC.

Keywords: Hepatocellular carcinoma (HCC), Circulating tumor cells (CTC), EpCAM, Sequencing

Background

Hepatocellular carcinoma (HCC) is a grim, heterogeneous disease with limited treatment options despite its enormous global impact as the third leading cause of cancer death worldwide [1]. Conventional liver imaging modalities for diagnosis and staging are imprecise and can result in underestimation of the true extent of disease, with microvascular invasion and multifocal tumors often identified incidentally at resection or transplant and associated with significantly poorer prognosis [2,3]. Translational research efforts to better understand the complex tumor biology of HCC, define biomarkers, and identify novel therapeutic targets are further limited by a scarcity of annotated, untreated tumor specimens, owing to the acceptance of radiographic diagnosis without tissue confirmation, the prevalence of liver-directed therapy before transplantation, and the risks associated with tumor biopsy in this population [4,5]. Non-invasive biomarkers for diagnosis and molecular characterization are urgently needed to overcome these pervasive challenges in HCC.

Circulating tumor cells (CTCs) in the peripheral blood are a biomarker of poor prognosis in multiple epithelial tumor types [6,7]. The CellSearch System (Veridex LLC, Raritan, New Jersey, U.S.A) is an FDA-cleared device for CTC detection using enrichment for cells in the blood expressing the epithelial cell adhesion marker (EpCAM) [6]. The absolute numbers of CTCs detected and changes on therapy have been associated with survival and treatment response in breast, colon, and prostate cancers [8-13]. Multiple small studies have examined CTCs in patients with HCC using EpCAM- and non- EpCAM-based enrichment methods, with detection rates ranging from approximately 30% to over 80% depending on methodology and population [14-17]. As in other epithelial tumor types, the detection of CTCs by CellSearch correlates with poor prognosis in HCC cohorts, including increased recurrence risk after resection and shorter overall survival [14,15].

In order to study CTCs as a biomarker in HCC, however, it is essential to establish that circulating epithelial cells in HCC populations are true tumor cells, rather than benign epithelial cells released into circulation as a consequence of the underlying inflammation or aberrant vasculature associated with liver disease. Though the detection of CTCs by CellSearch is extremely rare in healthy volunteers or patients with benign conditions [6,10], there is limited data describing the incidence of circulating EpCAM-positive epithelial cells in the context of cirrhosis, viral hepatitis, or other causes of liver injury, conditions present in the majority of patients with HCC [14].

Beyond detection and enumeration, isolation of CTCs in cancer patients holds great promise as a “liquid biopsy”, a non-invasive means of accessing real-time tumor tissue in the metastatic state for molecular profiling. Array comparative genomic hybridization has demonstrated concordance of characteristic copy number aberrations between CTC-derived DNA and archival primary tumor samples in breast, colon, prostate, and lung cancer [12,18-20]. Next-generation sequencing technologies now have the ability to sequence very small amounts of input DNA with high accuracy [21,22]. Illumina MiSeq technology can detect characteristic driver mutations in single CTCs derived from patients with metastatic colorectal cancer, concordant with the mutational profile of paired primary tumor specimens [18]. To date, the feasibility of efficient CTC isolation and molecular profiling, e.g. next-generation DNA sequencing, has not been reported in HCC.

We conducted this study to determine the proportion of metastatic HCC patients with detectable circulating EpCAM-positive epithelial cells using the CellSearch System, compared to a relevant control cohort of patients with liver disease, hypothesizing that circulating EpCAM-positive cells are actual tumor cells rather than benign epithelial cells. To characterize their prognostic significance, CTC levels were examined for association with clinical covariates including alpha-fetoprotein (AFP) levels, the presence of vascular invasion, and overall survival. To explore the potential for CTCs to serve as a source of tumor DNA for genomic profiling in HCC, next-generation sequencing using a targeted cancer gene panel was performed using whole genome-amplified DNA derived from pooled purified CTCs, along with DNA from paired archival, paraffin-embedded tumor tissue and peripheral blood mononuclear cells when available.

Methods

Study design

This pilot study was a non-therapeutic, minimallyinvasive biomarker study. The trial was approved by the UCSF Committee on Human Research. All patients provided written informed consent for specimen collection and genetic testing of tumor and germline DNA. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

The primary endpoint was incidence of CTCs detected in metastatic HCC patients compared to a control cohort with NMLD. Secondary endpoints were enumeration of CTCs in each cohort, association with clinical and pathologic characteristics including alpha fetoprotein (AFP) level, tumor vascular invasion, and etiology of liver disease in the HCC cohort, and association with overall survival in the HCC cohort. An exploratory endpoint was to describe performance of and somatic mutations identified by next-generation sequencing of CTC whole-genome-amplified DNA along with paired tumor and germline DNA when available.

Patient selection

HCC patients were recruited at the UCSF Helen Diller Family Comprehensive Cancer Center. Principal inclusion criteria were: radiographic [4] or histologic diagnosis of American Joint Committee on Cancer (AJCC) stage IV HCC; ≥ 6 weeks post biopsy, surgery, liverdirected interventions, or other invasive procedures; no prior systemic therapy or ≥ 4 weeks since last dose of sorafenib or other systemic therapy for advanced HCC. Non-malignant liver disease (NMLD) control cohort patients were recruited at the UCSF Gastroenterology and Liver Disease Clinic. Principal inclusion criteria were: diagnosis of active hepatitis of any etiology plus clinical or pathologic diagnosis of cirrhosis or hepatic fibrosis (any stage); no evidence liver tumor on ultrasound or cross-sectional imaging within 6 months; AFP ≤ 20 ng/mL within 6 months; ≥ 6 weeks post biopsy, surgery, or other invasive procedures; no prior history of HCC.

Specimen collection

Approximately 30 mL of whole blood was obtained from study subjects at a single time-point. For HCC patients with available archival tumor tissue from prior biopsy or resection, approximately five 10-micron sections of formalin-fixed, paraffin-embedded (FFPE) tumor along with a matching H&E slide were collected from the pathology files of the University of California, San Francisco. Banked frozen aliquots of peripheral blood mononuclear cell (PBMC) were obtained when available from HCC cohort patients.

Circulating tumor cell enumeration

CTCs were isolated from 7.5 mL whole blood and enumerated using the CellSearch System (Veridex LLC, Raritan, NJ) [6-8]. Briefly, specific antibodies to EpCAM were used to enrich for epithelial cells. A mixture of fluorescently-labeled monoclonal antibodies to cytokeratin and the nuclear dye DAPI were used to select for nucleated, keratin-positive cells. CTCs were defined as nucleated, EpCAM-positive cells that stain positive for cytokeratin and negative for leukocyte common antigen, CD45 [6]. Labeled cells were enumerated using semiautomated fluorescence-based microscopy. Analysis was performed by a trained technician blinded to diagnosis (HCC versus NMLD).

Immunoenrichment and fluorescence-activated cell sorting (IE/FACS)

A novel EpCAM-based immunoenrichment (IE)/fluorescence- activated cell sorting (FACS) procedure has been developed to isolate purified CTCs without contamination from normal blood cells and has demonstrated correlation with CellSearch System CTC enumeration [12,19,23]. For patients found to have > 10 CTCs in 7.5 mL of whole blood by CellSearch System, IE/FACS was then performed to isolate purified CTCs as has been previously described [12,24]. Briefly, approximately 15– 20 mL of whole blood was incubated with immunomagnetic particles coated with two different monoclonal antibodies to EpCAM, one conjugated to magnetic particles and the other to a fluorophore. FACS was used to isolate nucleated, EpCAM-positive, CD45-negative cells.

Whole genome amplification (WGA)

A ligation-adaptor method of WGA was performed on whole cell lysates from pooled CTCs isolated by IE/ FACS using a GenomePlex whole genome amplification kit (WGA4, Sigma-Aldrich) according to the manufacturer’s instructions [12,25]. DNA was randomly fragmented and converted to polymerase chain reaction (PCR)-amplifiable library molecules flanked by universal priming sites. PCR amplification of library molecules was performed using universal oligonucleotide primers.

DNA extraction from tumor tissue and peripheral blood mononuclear cells (PBMC)

Tumor-containing FFPE sections were identified and marked by a hepatopathologist (KE). DNA was extracted from FFPE sections as well as from banked PBMC using QIAmp kits (Qiagen) according to the manufacturer’s instructions. DNA concentration was quantified using PicoGreen.

Ion semiconductor NGS

Sequencing of DNA extracted from CTCs, FFPE, and PBMC was performed by TMB in the Spellman Laboratory at Oregon Health Sciences University. From each sample, 10 ng DNA was PCR-amplified using AmpliSeq Cancer Panel Primer Pools and Library Kit 2.0 to generate 190 multiplexed amplicons (representing 46 cancer-related genes) [21]. Up to 11 barcoded samples were multiplexed on Ion 318 chips. Sequencing was performed on a Personal Genome Machine (PGM) sequencer (Ion Torrent) using the Ion PGM 200 sequencing kit. Torrent Suite software version 4.0.1 was employed to analyze read counts and quality. Variant Caller software version 4.0.1 identified variants. Coverage Analysis software version 4.0.1 determined target coverage. To minimize false positives, variants were required to have sequencing depth of at least 20x, an allele frequency of 5 percent, and not be present in any of the 3 PBMC samples sequenced. Variant calls were filtered against the Single Nucleotide Polymorphism Database (dbSNP) version 132, using the software ANNOVAR. Protein-altering variants were predicted by Mutation Assessor version 2 (http://mutationassessor.org).

Statistical analysis

Based upon the a priori hypothesis that approximately 50% of the HCC cohort and none of the NMLD cohort would have detectable CTCs by CellSearch, the planned sample size for this pilot study was 20 patients with metastatic HCC and 10 patients with NMLD, to permit estimation of proportion of detectable CTCs with 95% confidence intervals (CI) as (0.30, 0.70) in the HCC cohort and (0.01, 0.26) in the NMLD cohort. The incidence and number of detectable CTCs were analyzed using frequency and proportions with 95% CI and compared between HCC and NMLD cohorts using the Wilcoxon-Kruskal-Wallis rank test. Cut-points of ≥ 1, ≥ 2, ≥ 3, and ≥5 CTCs/7.5 mL were examined based upon published literature in HCC and other tumor types [8,10,14,15]. Wilcoxon-Kruskal-Wallis rank testing was also used to determine association between the presence of detectable CTCs by CellSearch System, AFP elevation using ≥ 400 ng/mL as an established prognostic cut-point [26,27], and the presence of vascular invasion (all binary variables). In the HCC cohort, overall survival was measured in months from date of CTC blood draw to the date of death with censoring at date of last known vital status if lost to follow-

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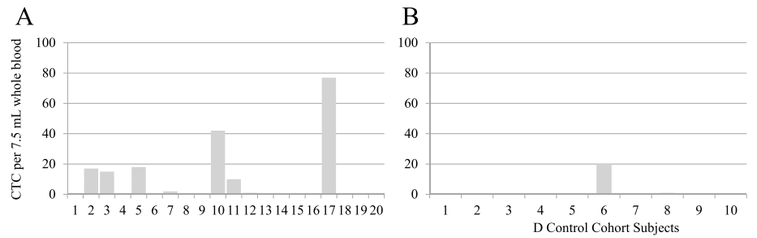

Figure 2 CTC detection and enumeration by CellSearch. Figure 2 depicts the CTC count per 7.5 mL whole blood by CellSearch in the HCC cohort **(A)** and NMLD control cohort **(B)**. *One patient in NMLD cohort who was removed for ineligibility due to new liver mass with portal vein thrombosis was found to have 20 CTCs per 7.5 mL peripheral blood.

eight somatic, non-synonymous variants were called mutations if a matching mutation has been described in liver cancer, if the variant shared the same amino acid residue as a COSMIC mutation in any cancer type, and/or if the variant allele frequency was greater than 5% but the variant was not a known SNP and not present in any PBMC sample [28]. Frameshift mutations were excluded from analysis due to known limitations of ion semiconductor sequencing to accurately detect frameshift mutations. Characteristic mutations in HCC (TP53, PTEN) were identified in CTCderived DNA from two cases. Figure 4 displays a summary of the somatic, non-synonymous mutations identified in CTC and FFPE tumor samples combined. A listing of all somatic, non-synonymous mutations (excluding frameshift) detected according to sample type is provided in Additional file 2. In one HCC case with matched CTC, FFPE tumor, and PBMC DNA, 8 SNPs were present and concordant in both FFPE tumor and PBMC DNA; 5 of these (63%) were detected in the CTC DNA. Neither was identified in the paired CTC DNA.

Discussion

The ability to detect and characterize malignant cells in circulation holds enormous promise as a biomarker in HCC, a grim cancer challenged by the inability of conventional noninvasive diagnostic and staging modalities to encompass its great clinical and biological heterogeneity, as well as by a scarcity of tumor tissue available for diagnostic or research purposes. In this study, at least one CTC was detected in 8/20 (40%) of patients with metastatic HCC, compared to 1/9 (11%) of eligible NMLD patients using the CellSearch System. Though the cut-point of ≥ 1 CTC/7.5 mL did not achieve significance between the two groups, a cut-point of ≥ 2 CTCs/ 7.5 mL was significant, positive in 7/20 (35%) HCC patients compared with none in the NMLD cohort ($p = 0.04$), consistent with prior reports [14,15]. The one eligible NMLD control patient with CTC count of 1/ 7.5 mL was subsequently found to have ultrasound findings suggestive of underlying tumor, although no formal HCC diagnosis was made, and thus he was not removed from the control cohort. Our findings confirm the limited existing data suggesting that circulating EpCAMpositive epithelial cells are rare in patients with nonmalignant liver diseases, and that EpCAM-positive cells in HCC patients are generally of tumor origin [14].

Corroborating the prognostic value of EpCAMpositive CTCs in other recent series [14,15], the detection of CTCs in the HCC cohort of this study was significantly associated with high AFP and the presence of vascular invasion, and there was a non-significant trend toward poorer overall survival in patients with detectable CTCs. These findings support the value of CTCs as a prognostic biomarker in metastatic HCC and suggest future potential roles for CTCs in treatment decisionmaking as

[a]Data from FFPE and PBMC DNA samples were combined for sequencing performance analyses (but not for genotype analyses) due to small sample size and similar observed coverage. NS = not significant. [b]PBMC samples (germline DNA) were excluded from variant analyses, n = 3.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
RKK developed study concept, design, and protocol, consented and enrolled patients, managed and analyzed data, and wrote manuscript. MJ-MM performed CTC and WGA assays and contributed to data analysis and writing. TMB performed sequencing and analysis of sequencing data. EAC performed DNA extraction and contributed to study design, analysis, and writing. JH participated in study design and performed statistical analysis. NS performed DNA extraction. KE reviewed and marked pathology specimens for tumor content. RMM assisted in patient consent, blood specimen collection, and study coordination. BH, EMW, and FYY identified and consented control cases. APV participated in study design and data analysis. JWP participated in study design, developed IE/FACS assay, and contributed to data analysis and writing. All authors read and approved the final manuscript.

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CI1000

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2009

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180 /

CI1000

CI1000

CI1000

CI1000

IVD

IVD 2014 260

IVD 35% 32%

42 38

IVD

IVD

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1. 180test/h
2. CV<2%@10uL
   CV<0.5%@50uL 1ppm
3.
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9.
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CI000

2009

CI1000

3 3

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CI1000

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2014 7 CI1000

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CI1000 2.

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5.

DFMEA

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2012 9

CI1000

CI000

1		180 t/h	
2		CV<5%	AFP TPOAB T4 CV ≤ 5%
3		r ≥ 0.99	AFP TPOAB T4 r ≥ 0.99
4		14min	
6		<1ppm	
7			
8			
9		4	
10		20 100	
11			Φ13×75mm Φ13×100mm
12		CV<2%@10uL CV<0.5%@50uL	
13		6 /7	6 /7
14		24	
15		2 8	24
16		100	
17		72	
18		37+/-0.1	
19		4	4
20			
21		<15 <30	

CI000

	ARCHITECT i2000SR i2000	Access2 (Access)	E601(E170)	CI1000
	abbott	Beckman	roche	Leadman
		ALP-APMMD	ECLIA	ALP-APMMD
	200test/h	100test/h	170test/h	180test/h
	15.6min	15min	18min	14min
	25	24	25	24
	100/500test	100test	100/200/test	100test
	6	6	2	6 7
	30	28	28	28
	135	60	150	100 20
	5	3	5	4
	50uL	5uL	10uL	5uL
		<1PPM		<1PPM
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CI1000 CI1000

60 10

20

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CI1000

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1. ROCHE

2. ABBOTT ARCHITECT

3. BECKMAN

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1 T3

ARCHITECT 10.5pmol/L

E170 13.0pmol/L 2

2 T3

E170 13.0pmol/L 2

E411 11.9pmol/L

2.

1.

1. T3 FT3

	T3	FT3
	0.8-2.0ng/mL	1.8-4.6pg/mL
	0.58-1.59 ng/mL	1.71-3.71pg/mL
IMMULITE	0.84-1.72 ng/mL	1.8-4.2 pg/mL

3.

4.

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1

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2. FT3 T3 TSH

	FT3	T3	TSH
	(Equilibrium dialysis)		WHO 2 rd IRP 80/558
	(Gravimetry)		WHO 2rd IRP 80/558
	Internal standard		WHO 2 rd IRP 80/558
ADVIA Centaur	USP	USP	WHO 3rd IRP 81/565

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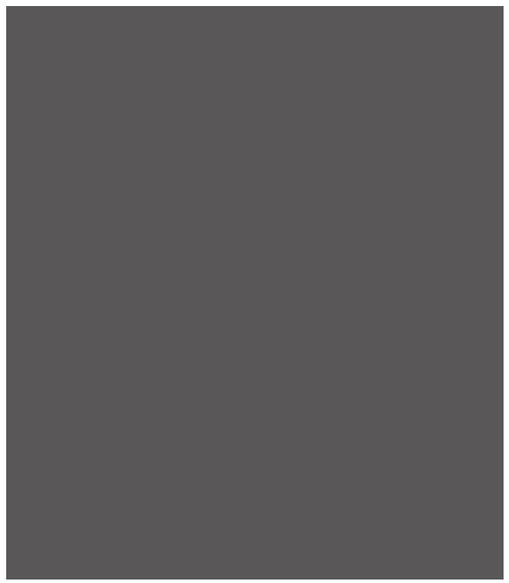

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AU680 C :1 10 R1+S

ALP ALP 1 1

37

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D :10 11 2 R2

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1.3 AU680 5 1 : ALP 2

405nm

405nm ALP

10

2 2

E 11 D E

11

1 ALP

A 0 1 R1

A 1 1

A

OD 0

OD

IVD

2015 37

2015

55

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6 11—16

16

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1	ALT)		(1) 36 16 20 (2) (3) 20 20		
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4	f-PSA)		(1) 28 8 20 (2) (3) 20 20		
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9	IgG		(1) 20 20 (2)		
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11	TP		(1) 2 (2) 40 10 1 2 1		
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13	ALP NPP -AMP NPP -DEA		(1) 2 (2) 40 10 1 2 1		
14	ALB)		(1) 2 (2) 40 10 1 2 1		
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NSE( )

(neuron-specific enolase NSE)

NSE

SCLC NSE NSE

(1) (SCLC) NSE 60-81% NSE
NSE
NSE 80% 80% 90% (NSCLC)
SCLC NSCLC NSE SCLC
NSE SCLC

(2) NSE 96% 100% NSE
NSE

(3) NSE NSCLC

CA50(CA50)

CA50

CA50 <20 μg/L
66.6% 88.2% 68.9% 88.5%
94.4% 70%

(1) CA50

(2) CA50

(3) CA50

SCC( )

TA-4 SCC

(1) SCC 83% 25% 75% 30%
89%

(2)

(3)

——PGI PGII

90 [4] PG

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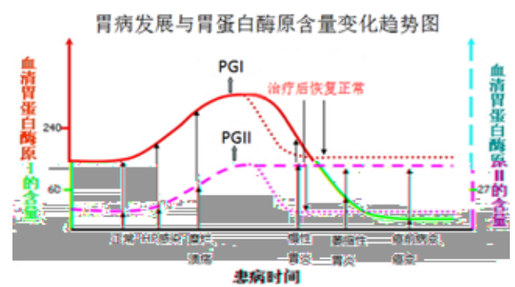
40% 2012



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cAMP

(Pepsinogen,PG) PG PGII[5] PGII

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PG HP

1994 HP

Helicobacterpylori HP 60 HP

HP 1.8% [6] PG HP

[2] HP —— ABC 1

—— —— —— 78%[7]

PG 1

PGI PGI/II

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		-	+
PG	-	A	B
	+	C	

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HP

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15 [3]

A
B
C

PG HP 1998
PGI/PGII
HP [8] HP PGI PGII
60%
HP HP

PG

PGI,PGII PGI
PGII PGI PGI/PGII PGII
PGI PGII
PGI/PGII 2.9±0.4
PGI
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0.7±0.3 Sipponen 1987
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Gritti 2000
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PGII
PGI/PGII
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[9] PGI

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PG

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PGI PGII .
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| 303 5230021

: , : 2007-2010 52 : 52 , 45 51 ; 31 11 ; 46 γ 24 IgG-κ 7 IgG-λ 6 IgA-κ 4 IgA-λ 3 IgM-λ 2 λ1 1 TP>80G/L 28 GLO>30g/L 36 , A/G<1.0 39 >2.7 mmol/L 12 ; 4.5% 76.5%(40.5%) :

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R733.3 [ ]A

The Laboratory Features Analysis of Multiple Myeloma

Liu TieNiu,Chen YaoPeng, Huang Li ,Qian Wu Xia , Huang GuiCai
NO.303 hospital of PLA NanNing GuangXi 530021

Abstract **Objectives:** To analyse the laboratory features of the patients wwith multiple myeloma . **Methods:** The records of 52 patients with MM in our hospital from 2007 to 2010 were retrospectively reviewed . **Results:** Of the 52 study patients with MM,45 of them have various degrees of hemopthisis;51 of them have growth of eruthrocutes sedimentation rate;31 of them have proteinuria,;11 of them have Bence-Jones protein; 46 of them have serum M protein in the serum electrophoresis test .Serum immunofixation electrophoresiswas performed in 24 sera samples, there were 7 cases of IgG-κ, 6 IgG-λ, 4 IgA-κ, 3- IgAλ, 2 IgM-λ, 1λchain and 1 natural. Serum total protein increased TP>80g/L in 28 samples, globulin level also increased in 36 cases,A/G <1.0 in 39 cases Serum calcium >2.7 mmol/L in 12samples. the proportion of Myeloma cells in bone marrow is 4.5% 76.5%(the average level is 40.5%). Conclusion because of the complicated laboratory tests ' results, The related laboratory comprehensive analysis may increase the veracity diagnosis of MM.

Keywords **Multiple Myeloma(MM); Laboratory diagnosis; Myeloma cells**

(Multipe myelama MM) , 10%[1] , MM 2007 -2010 52 MM

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1 1 2007 -2010
52 , 29 , 23 ;
1.27 1 35 85 61.5
[2]

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2.1 52
7 , 33 29 , 4
4 , 1
14 , [3]
4 105mm/h
4 0-15 mm/h, 0-20 , 4
mm/h 1

2.2 52 31 ,
±~+++ 11

2.3 1
46 γ 24
IgG-κ 7 IgG-λ 6 ,
IgA-κ 4 IgA-λ 3 IgM-λ 2 ,
λ1 1 IgE 41 52 7
IgG 28 IgA 9 IgM
2 IgE 1 IgG A M 1

2.4 52
TP>80g/L 28 GLO>30g/L 36 , A/
G<1.0 39 2.36±0.5 mmol/L
>2.7 mmol/L 12 :2.10-2.70 mmol/L) [5]
[6]

2.5 52
1.2% 95%(40.5 %) , >15%
31 (%) , <15% 21 7

1.4%-5.6%

3

B
(Ig), M

MM
MM
52

3.1
MM
, 1
[3]
105mm/h
4
, 4
65.6% 82.5% 48.8% 79.5%

MM
M
β β-γ
γ
M
[9]
52 46 γ
,
[10]
IgG
IgA IgM κ λ IgE IgD
IgG
IgA IgM IgD IgE κ λ

3.2 MM
60% 31/52
[4] 52
11 ,

3.3 A/G
IgG IgA IgM
IgD IgE ,
κ λ
MM
[8]

3.4 A/G
52 52

MM κ λ TP>80g/L 28 GLO>30g/L 36 ,
A/G<1.0 39
[7]

MM ,
15 20% MM

, MM ,
[11 12]
, ,
ALB<40g/L mmol/
L=(mmol/L-0.025* g/L+1.0)
>2.7mmol/L 12
>2.7mmol/L 17

40g/L
0.025[13] mmol/L=
mmol/L-0.025* g/L
1.12mmol/L-1.23 mmol/L
[14]

,

(Nasopharyngeal carcinoma NPC)

-18 (Interleukin-18 IL-18)

γ-

(IFN-γ)

[1, 2]

IL-18

IL-18

IL-18 11 11q22.2-q22.3

6 5

IL-18 (SNPs)

[3-5] IL-18

- (PCR-

RFLP) IL-18

1

1.1

190

135 55 48.3±8.1

200

140 60 46.5 ± 7.6

1.2

1.2.1 DNA 3ml

EDTA-K2

DNA -70

1.2.2 PCR Genbank

(NT033899.7)

IL-18 -607

DNA 5'-

CCCTCTCCCCAAGCTTACTT-3' 5'-

TTCAGTGGAACAGGAGTCCA-3'

IL-18 -137 DNA

5'- TTGTAAC ATTGTAGGAATTACC-3'

5'- ATGTAATATCACTATTTTCATGAGA

-3' IL-18 PCR 25μl

10×PCR 2.5μl 2.5mmol/L dNTPs 2.0 μl

20pmol DNA 2.0μl TaqDNA

1.25U 25μl

(Bio-Rad) 94 5 min

35 94 30 s 60 45 s

72 55 s 72 5 min

1.2.3 PCR 3

μl 2 U Mse I BfuC I

Biolabs IL-18 -607 -137

37 3 8 %

EB

1.3

SPSS11.5

Hardy-Weinberg

χ^2 OR

95% CI P<0.05

2

2.1 IL-18

IL-18 -137 G/C

PCR 256 bp

BfuC I 3 CC

256 bp 1 GC 256 bp 229 bp 27

bp 3 27 bp GG (229 bp

27 bp 2) (1) IL-18 -607 C/A

PCR 137 bp

Mse I 3 AA 91

bp 46 bp 2 46 bp CA 137

bp 91 bp 46 bp 3 CC 137 bp 1

(2) PCR

Genbank (NT033899.7) 3-4

2.2 IL-18

IL-18

Hardy-Weinberg

χ^2 IL-18 -137

χ^2=9.895

P=0.002 C

G 1.730

OR=1.730 95%CI 1.227 2.439 IL-18 -607

C/A P>0.05

1

2.3 IL-18

SHEsis IL-18 -137

G/C -607 C/A 2

IL-18 -137 G/C -607

C/A (|D'|=

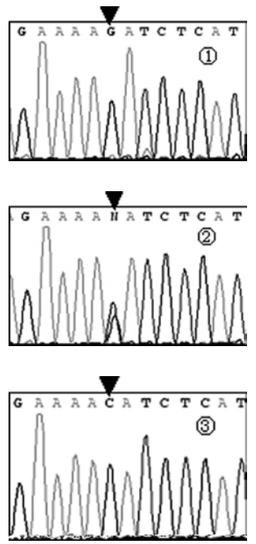
0.951) 3

χ^2=9.530

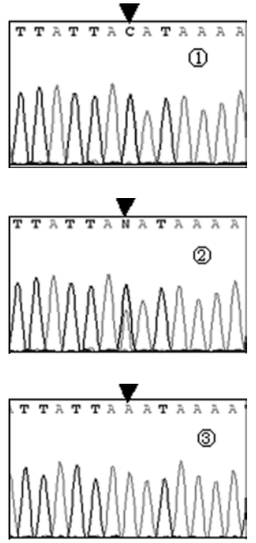
P=0.009 -137C/-607A

OR=1.699 95%CI 1.174 2.457

2



3 IL-18 -137 G/C GG GC CC



4 IL-18 -607C/A CC CA AA

1, 3: GC 2, 4: GG 5, 6: CC M:

图 1 IL-18 基因 -137 G/C 多态性 8 % 的聚丙稀酰胺凝胶电泳图

1, 6: CC 2, 3, 5: AA 4: CA M:

图 2 IL-18 基因 -607 C/A 多态性 8 % 的聚丙稀酰胺凝胶电泳图

1 IL-18

	NPC n = 190 (%)	n = 200 (%)	χ^2	P
IL-18 -137 G/C				
GG	102 (53.7)	139 (69.5)	10.359	0.006
GC	74 (38.9)	52 (26.0)		
CC	14 (7.4)	9 (4.5)		
G	278 (73.2)	330 (82.5)	9.895	0.002
C	102 (26.8)	70 (17.5)		
IL-18 -607 C/A				
CC	40 (21.1)	56 (28.0)	2.535	0.282
CA	97 (51.1)	93 (46.5)		
AA	53 (27.9)	51 (25.5)		
C	177 (46.6)	205 (51.3)	1.701	0.192
A	203 (53.4)	195 (48.7)		

2 IL-18

IL-18	NPC 2n = 380 (%)	2n = 400 (%)	(95%)	P
-137 G/-607 C	174 (45.8)	203 (50.8)	1.000 ( )	
-137 G/-607 A	107 (28.2)	129 (32.3)	0.968 (0.698-1.341)	0.844
-137 C/-607 A	99 (26.1)	68 (17.0)	1.699 (1.174-2.457)	0.005
-137 C/-607 C	0 (0.0)	0 (0.0)		

3

-607 A

Nong [9] IL-18

IL-18 -137 G/C

-137 C

IL-18 -137 C/-607 A

IL-18

IFN-γ TNF-α Sáenz-López [10] IL-18 -137 G/C -607 C/A FasL NK

IL-18 IL-18

IL-18 IL-18

IL-18 - IL-18

[6, 7] IL-18 IL-18 -137 G/C

11 6 5 C

IL-18 P<0.05

IL-18 IL-18 IL-18 -137 G/C -607 C/A (|D'|= 0.951)

IL-18 3

χ^2=9.530 P=0.009 -137 C/-607 A

IL-18 OR=1.699

Farjadfar [8] IL-18 95%CI 1.174 2.457 IL-18 -137 G/C -137 C/-607 A

IL-18 -607 C/A -137 C

-607 A

-607 A IL-18 Nong [3]

IL-18 -137 G/C

-137 C/-607 A

-137 C

IL-18

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TAP

(TAP)

TAP TAP

TAP AFP CEA

AFP CEA

γ - α1-

TAP

CTCs

Circulating Tumor Cells, CTCs

1896 Ashworth

CTCs

1 CTCs 10mL

2

3 CTC

CTCs

CTCs mRNA CTCs

CTCs

Tumor abnormal protein (TAP)

Tumor abnormal protein (TAP) is abnormal carbohydrate glycoprotein of all kinds of normal tissue cells during malignant transformation. A lot of research shows that TAP can be detected in the peripheral blood when it achieves a certain amount. TAP is closely related to tumor genesis, development, metastasis, and tumor prognosis.

TAP detection is a combination of tumor markers detection technique. It can detect the tumor markers in the same reaction system, contains existing items and other that still unable to be detected (including AFP, CEA, carbohydrate antigen, transferrin, alkaline phosphatase, γ-glutamyl transferase, human chorionic gonadotropin, α 1- antitrypsin and prostatic acid phosphatase). Highly enhance detection signal, not only greatly improve the sensitivity and accuracy of detection, but also detect more types of cancer. TAP is suitable for the detection of cancer screening, early detection of cancer, therapeutic effect evaluation and monitoring and evaluation of prognosis and recurrence in patients with metastatic cancer.

Circulating tumor cells (CTCs)

Circulating tumor cells (CTCs) are rare malignant cells found in the peripheral blood that originate from the primary tumor or metastatic sites.
Ashworth proposed the concept of circulating tumor cells for the first time in 1896. The majority of CTCs are dead in a short time. Only very few tumor cells with high viability and high potential of metastasis can survive in the circulation system and develop to metastases under certain conditions. Therefore, the detection of tumor cells in peripheral blood may indicate tumor metastasis occur.

The characteristics of circulating tumor cells:
(1) Scarcity: the number of CTCs in blood is extremely low.
(2) Specificity: the species classes and expression of biomarkers are different between different tumor cells.
(3) Atypical cells: tumor cells usually has a high ratio nuclear mass. But tumor cells, especially CTCs present a typical morphology due to the influence of the circulation of blood and some of the stress reactions.

With the development of molecular biology techniques, the detection sensitivity and specificity of CTCs will continue to improve. At present the single genome level and mRNA expression analysis of CTCs will help to better understand the essential of CTCs. As a noninvasive detection method, the detection of CTCs can be effectively applied to early diagnosis and rapid assessment of chemotherapeutic drugs and individualized treatment, clinical drug screening, drug resistance detection, monitoring of tumor recurrence, targeted therapy of cancer drugs, drug development, etc.