

Cancer Panel Analysis of CTC in Breast Cancer Patients*

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BACKGROUND & PURPOSE

Circulating Tumour Cells (CTCs) can be released from the primary tumor into the bloodstream and they may colonize distant organs and give rise to metastasis.

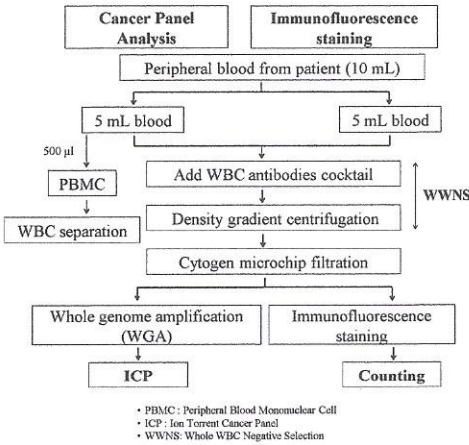
The isolation of CTC has been a major technological challenge due to their rareness, therefore overcome of challenge can promote the application of CTC in the routine medical practice.

Here, we tried to enrich ample amount of CTC for cancer panel analysis, using microfabricated chip & anti-WBC antibody complex.

Liquid biopsy using CTC may provide molecular profiling of tumor, which can be useful for determination of personalized cancer therapy according to the molecular profiling of individual patient during the process of tumor diagnosis and treatment. Here, we enriched CTCs from breast cancer patients and successfully analyzed genomic information of those.

MATERIALS AND METHODS

<CTC isolation process for cancer panel analysis>

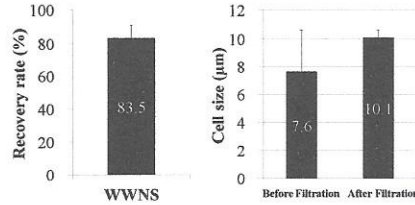


<Clinical features of 19 breast cancer patients>

Female	N (%)	
Age (years)	Median	45
	Range	28 - 56
ER/PR/HER	++ -	12 (63.2)
	+- -	3 (15.8)
	- - -	4 (21.1)
AJCC/TMN stage	II A	6 (31.6)
	II B	5 (26.3)
	II C	1 (5.3)
	III A	5 (26.3)
	III B	0 (0)
	III C	2 (10.5)
Histologic grade	2	16 (84.2)
	3	3 (15.8)
Nuclear grade	2	16 (84.2)
	3	3 (15.8)

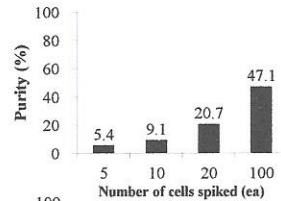
* ER: estrogen receptor / PR: progesterone receptor / HER: human epidermal growth factor receptor
 * AJCC: the American Joint Committee on Cancer / TMN: Tumor Metastasis lymph Nodes

RESULTS

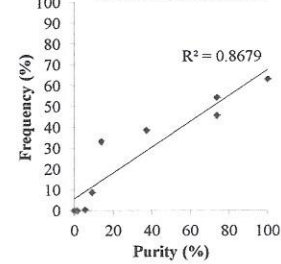


Recovery rate of CTC capture obtained from GFP-H358 cells spiked into normal blood

Comparison of cell size after CTC microchip filtration using cancer patient's blood



Purity after entire enrichment process at four different cancer cell loads



PIK3CA mutation (COSM 125370) detection from MCF7 using Ion torrent cancer panel

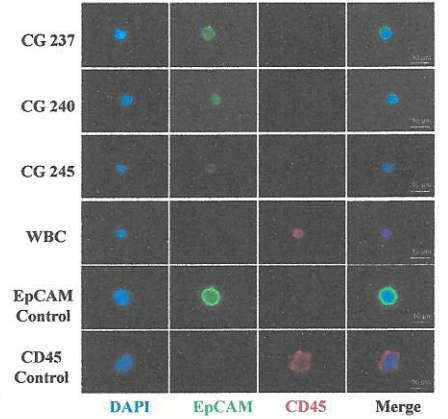
Average DNA Concentration	593.5 ng/µl
Range	89 - 688 ng/µl
Average Total DNA	30.7 µg
Range	4.9 - 37.8 µg
Average Purity	14.2 %
Range	0 - 100 %

Whole Genome Amplification results of 11 breast cancer patients

<ICP analysis of 11 breast cancer patients>

Patient ID	TMN stage	EpCAM (+) No.	Gene	COSM No.	Allele (Gene) Frequency
CG 188	II B	1	IDH2	33733	4
			TP53	43753	2.7
			NRAS	564	21
			IDH1	28747	2
			PDGFRA	22413	57.6
CG 194	III A	1	EGFR	27110	1.8
			PTEN	23626	2.3
			PTEN	4994	2.3
			PTEN	4990	2.3
			STK11	25851	4.9
CG 195	II B	1	TP53	44547	1.7
			STK11	25851	18.1
			MLH1	26085	51.6
			PIK3CA	14052	1.8
			STK11	25851	3.8
CG 197	II A	3	CDKN2A	14253	53.5
			IDH2	33733	2
			KIT	28026	48.2
CG 199	III C	1	SMARCB1	1090	35.1
			PTEN	23626	2.8
			PTEN	4994	2.8
CG 200	III C	3	PTEN	4990	2.8
			PTEN	4990	2.8
			PTEN	4990	2.8
CG 202	III A	19	X	X	
CG 203	II B	6	HRAS	249860	84.6
CG 204	II B	7	KIT	21983	52.9

<Enumeration of CTC from cancer patients>



<ICP analysis of CTC and WBC pair from 8 breast cancer patients>

Patient ID	WBC gene (COMS No.)	CTC gene (COMS No.)	CTC specific gene (COMS No.)
CG 237	MLH1 (26085)	MLH1 (26085)	
	RB1 (1042)	STK11 (25851)	STK11 (25851)
	PDGFRA (22413)	PDGFRA (22413)	
CG 238	PTEN (23626)		
	PTEN (4994)		
	PTEN (4990)		
	STK11 (25851)		
CG 239	HRAS (249860)	HRAS (249860)	
	VHL (14310)	VHL (14310)	VHL (14310)
	PDGFRA (22413)	PDGFRA (22413)	
CG 240	MET (710)	MET (710)	
	HRAS (249860)	HRAS (249860)	
	STK11 (21378)	STK11 (21378)	
CG 242	MLH1 (26085)	MLH1 (26085)	
	PDGFRA (22413)	PDGFRA (22413)	
CG 243		STK11 (25851)	STK11 (25851)
	PDGFRA (22413)	PDGFRA (22413)	
		MET (710)	MET (710)
CG 244		PTEN (23626)	PTEN (23626)
		PTEN (4994)	PTEN (4994)
		PTEN (4990)	PTEN (4990)
		PTEN (5000)	PTEN (5000)
		RET (966)	RET (966)
CG 245	PTEN (23626)		
	PTEN (4994)		
	PTEN (4990)		
	STK11 (25851)		

CONCLUSION

We provide a new approach to obtain enough amounts of CTCs for molecular analysis. And this new approach can be an applicable tool of serial liquid biopsy in breast cancer treatment.

REFERENCES

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