Detection of androgen receptor in circulating tumor cells (CTCs) from patients with prostate cancer

Jeong-Mi Moon[1], Young Hun Kim[1], Cham Han Lee[1], Byung Hee Jeon[1], Myoung Shin Kim[1], U-Syn Ha[2]

[1]Cytogen, Inc., Seoul, Korea
[2]Department of Urology, Seoul St. Mary's Hospital, The Catholic University of Korea

http://www.cytogenlab.com/

SMART BIOPSY™ SYSTEM

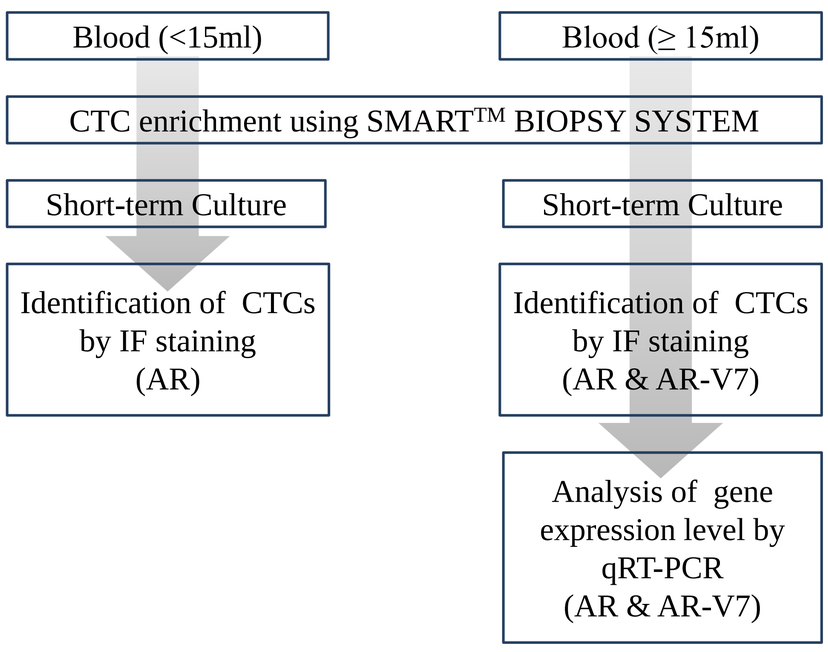
Abstract

Prostate cancer is a common cause of cancer mortality in men. The androgen receptor (AR) signaling pathway plays an important role in the progress as well as metastasis of the prostate cancer. Thus, evaluation of AR expression can be a useful and significant tool for prognosis prediction and treatment selection in prostate cancer patients. While the tissue biopsy is performed only in a low proportion of cancer patients, the detection of circulating tumor cells (CTCs) can be applied for majority of cancer patients, therefore liquid biopsy using CTC can be considered as an alternative biopsy tool for patients with cancer.

Here, we suggest the isolation of CTCs and the analysis of AR as an alternative to tumor tissue biopsy. Fifteen milliliters of blood samples were collected in ACDA tubes from 32 patients with prostate cancer and processed by size-based filtration using Cytogen's CTC isolation platform. The CTCs isolated from 5 ml of blood were immunofluorescent-stained for cytokeratin, CD45 and androgen receptor. The CTCs from 10 ml of blood were cultured, and then analyzed for mRNA level of AR by quantative RT-PCR. CTCs were detected in 31 of 32 patients (96.9%, range 1 – 138), and the AR positive CTCs were detected in 30 patients of 32 patients (93.8%). And, mRNA levels of AR were evaluated in cultured CTCs by qRT-PCR. These results suggest that the isolation and culture of CTCs can be a substitute method for tumor tissue biopsy, and may provide clinical applications.

Materials & Methods

◆ Research Flow-Chart



◆ IF staining Condition

- DAPI / CD45 / pan-Cytokeratin / AR
- DAPI / CD45 / pan-Cytokeratin / AR-V7

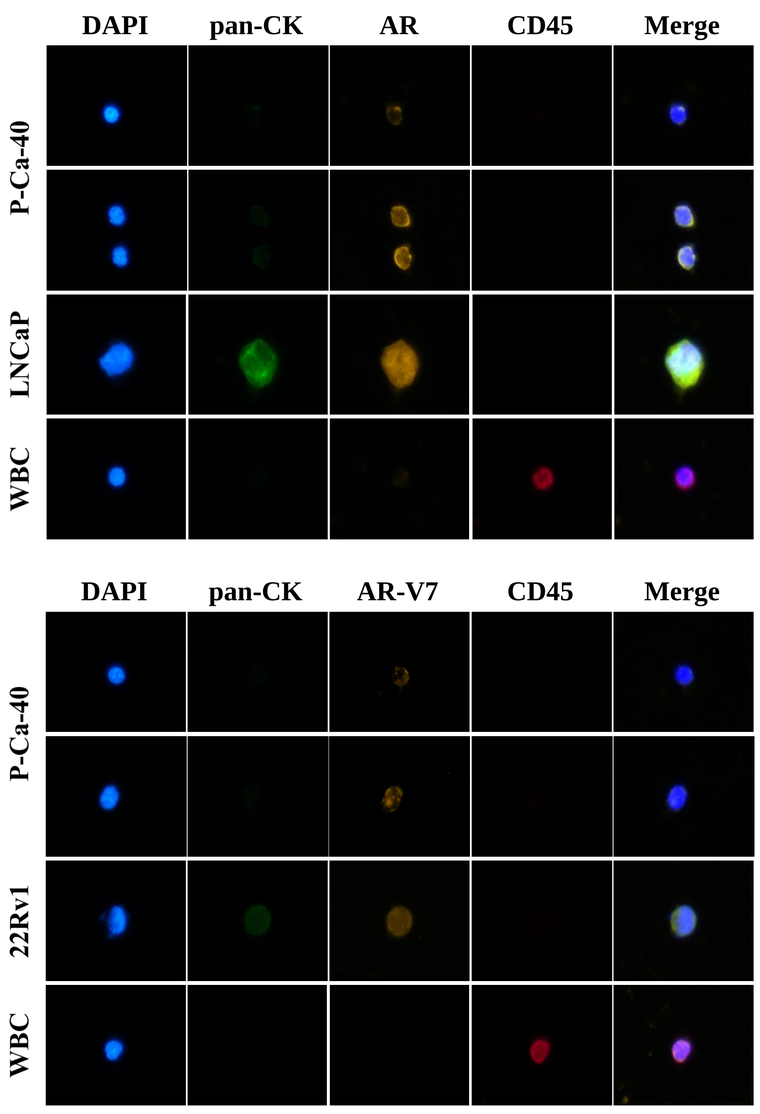
Results

◆ CTC count of Prostate Cancer patients

No.	ID	TNM stage	Initial CTC # 5ml	
			AR	AR-V7
1	P-Ca-1	pT3bN0M0	25	-
2	P-Ca-2	pT3aN0M0	8	-
3	P-Ca-3	pT4N0M0	69	-
4	P-Ca-4	pT3bN0M0	24	-
5	P-Ca-5	pT3bN0M0	32	-
6	P-Ca-6	pT3bN1M0	148	-
7	P-Ca-7	pT2cN0M0	28	-
8	P-Ca-8	T3bN1M1b	8	-
9	P-Ca-9	T3bN1M1b	3	-
10	P-Ca-10	pT3bN1M0	35	-
11	P-Ca-11	pT3aN0M1b	11	-
12	P-Ca-12	pT3aN0M0	50	-
13	P-Ca-19	pT2cN0M0	5	-
14	P-Ca-20	T3bN1M0	2	-
15	P-Ca-21	pT4N1M0	22	-
16	P-Ca-22	pT2cN0M0	20	-
17	P-Ca-23	pT3aN0M0	22	-
19	P-Ca-25	pT4N1M0	10	-
20	P-Ca-26	pT4N1M1b	34	10
21	P-Ca-27	pT3aN1M1a	8	-
22	P-Ca-28	pT2cN0M0	12	3
23	P-Ca-29	-	1	7
24	P-Ca-30	-	2	-
25	P-Ca-31	-	7	6
26	P-Ca-32	-	1	5
27	P-Ca-33	-	4	4
28	P-Ca-34	-	9	7
30	P-Ca-36	-	7	4
31	P-Ca-37	-	1	7
32	P-Ca-38	-	1	0
33	P-Ca-39	-	2	1
34	P-Ca-40	-	13	9
35	P-Ca-41	-	0	2
36	P-Ca-42	-	2	0
37	P-Ca-43	-	1	0
38	P-Ca-44	-	0	1
39	P-Ca-45	-	3	1
40	P-Ca-46	-	1	4
41	P-Ca-47	-	50	8
42	P-Ca-48	-	3	5

- : Not analyzed.

◆ Representative IF images



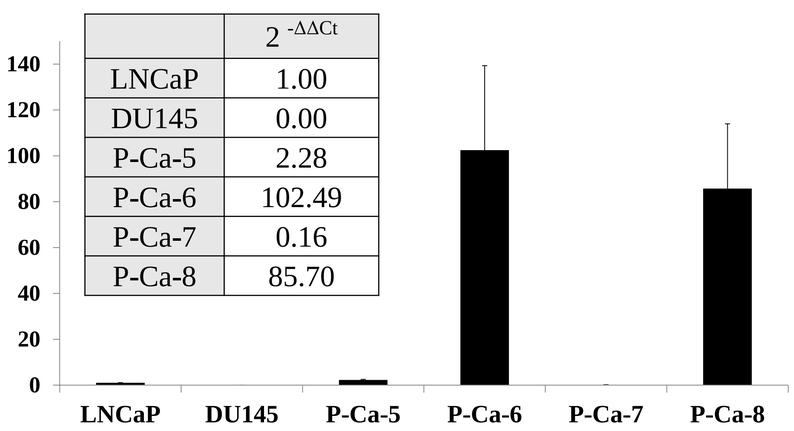
◆ Relation between CTC count and Cancer stage

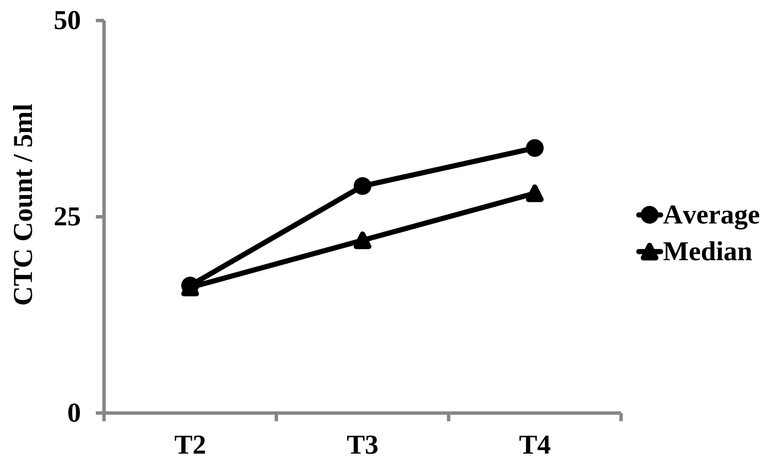
(AR Group)

T stage of TNM	Case number	Range	CTC Count / 5ml	
			Average	Median
T2	4	5 – 28	16.25	16
T3	13	2 – 148	28.92	22
T4	4	10 – 69	33.75	28

◆ Total AR expression level

	$2^{-\Delta\Delta Ct}$
LNCaP	1.00
DU145	0.00
P-Ca-5	2.28
P-Ca-6	102.49
P-Ca-7	0.16
P-Ca-8	85.70

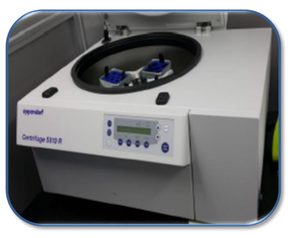


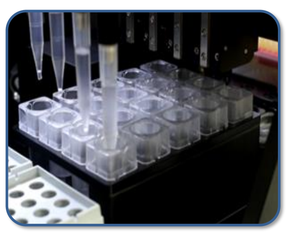


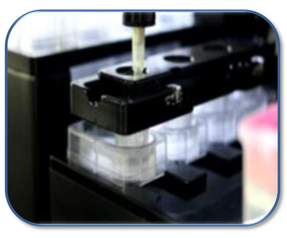
SMART BIOPSY™ SYSTEM

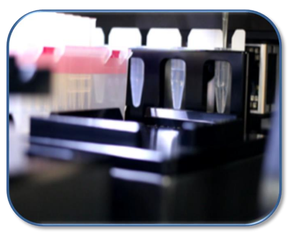
◆ CTC enrichment process


Blood collection


Pre-processing


Size-based gravity flow filtration




Retrieval of CTCs

◆ SMARTBIOPSY™ Cell Isolator

Smart Biopsy™ Cell Isolator enriches intact rare cells from human blood and/or body fluid using HDM chip. (High density microporous chip)

By size-based filtration, it captures viable cells, which can be useful for downstream application including genomic analysis, immunofluorescent staining, and culture.

Conclusion

The pilot study of prostate cancer CTCs was performed to analyze the relationship between CTCs and prostate cancer using IF staining (pan-CK / AR / AR-V7) and qRT-PCR.

The following facts have been identified:

1. The number of CTCs increased according to the TNM stage.
2. Total AR expression was changed in the prostate cancer CTCs

Additionally, AR-V7 gene was identified in the prostate cancer patients' CTCs. However, clinical facts were not considered in the study, such as therapeutic history, etc.

Therefore, to reveal the relationship between clinical information and CTCs information (number/5ml, AR and AR-V7 expression), we will perform further study.

CytoGen Advanced Laboratory Service Company