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

Young Hun Kim[1], Myoung Shin Kim[1], Cham Han Lee[1], Jeong Mi Moon[1], Hye Seon Lee[1], Mi So Choi[1], Ji-hyun Uh[1], Yun Gyu Jeong[1], Byung Hee Jeon[1], U-Syn Ha[2]

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

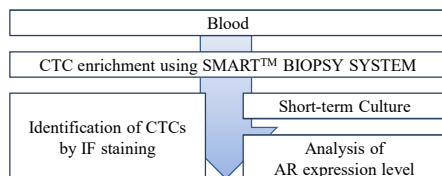http://www.cytogenlab.com/

SMART BIOPSY™ SYSTEM

Abstract

Prostate cancer is the most common cancer among men, and androgen receptor (AR) signaling is important on tumor progression and survival as well as metastasis. Evaluation of androgen receptor expression is a useful and significant tool for prediction of prognosis and treatment selection in prostate cancer patients. However, tumor biopsy in cancer patient is not always possible.

Here we suggest the isolation of CTCs and the analysis of androgen receptor 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 from 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 androgen receptor by real-time PCR. CTCs were detected in 31 of 32 patients (96.9%, range 1 – 138), and the expression of androgen receptors was detected in 30 patients (93.8%). 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

Blood
↓
CTC enrichment using SMART™ BIOPSY SYSTEM
↓
Identification of CTCs by IF staining
→ Short-term Culture
→ Analysis of AR expression level

◆ Study Design

1. 5ml : IF staining (AR)
2. Blood Volume <15ml : IF staining (AR & AR-V7)
3. ≥ 15ml : IF staining (AR & AR-V7) / qRT-PCR

◆ IF staining Condition

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

Results

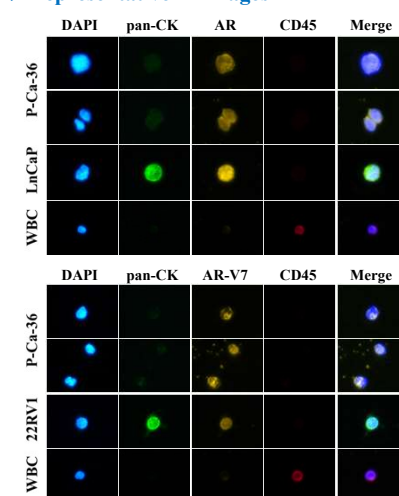### ◆ CTC count of Prostate Cancer patients

No.	ID	TNM stage	Initial CTC # / 5ml AR	Initial CTC # / 5ml 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	-
18	P-Ca-25	pT4N1M0	10	-
19	P-Ca-26	pT4N1M1b	34	10
20	P-Ca-27	pT3aN1M1a	8	-
21	P-Ca-28	pT2cN0M0	12	3
22	P-Ca-29	-	1	7
23	P-Ca-30	-	2	-
24	P-Ca-31	-	7	6
25	P-Ca-32	-	1	5
26	P-Ca-33	-	4	4
27	P-Ca-34	-	9	7
28	P-Ca-35	-	0	0
29	P-Ca-36	-	7	4
30	P-Ca-37	-	1	7
31	P-Ca-38	-	1	0
32	P-ca-39	-	2	1

- : Not analyzed.

◆ Total AR expression level

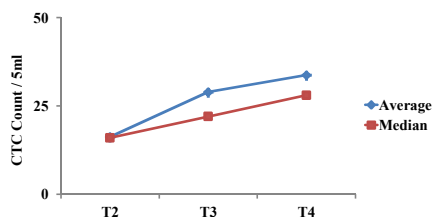|	$2^{-\Delta\Delta Ct}$
LnCaP	1.00
DU145	0.00
P-Ca-5	2.28
P-Ca-6	102.49
P-Ca-7	0.16
P-Ca-8	85.70
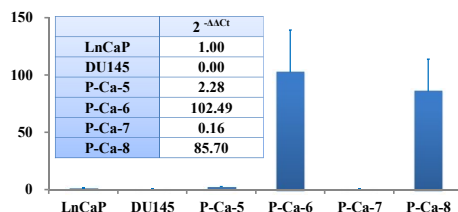
◆ Representative IF images

◆ Relation between CTC count and Cancer stage

(AR Group)

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

SMART BIOPSY™ SYSTEM

◆ CTC enrichment process

Blood collection   Pre-processing   Size-based gravity flow filtration   Retrieval of CTCs
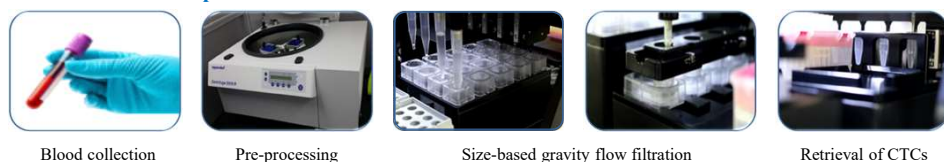
◆ SMARTBIOPSY™ Cell Isolator

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) 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.