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Distinct Expression of Surface and Genetic Biomarkers in Prostate Cancer Cell Lines

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Abstract. *Background/Aim: Surface biomarkers, such as CD44 and CD133, have been demonstrated to be expressed in prostate cancer cells, and our previous study has shown that prostate cancer cell lines could be divided into three groups according to the single and combined expression pattern of CD44 and 133. In order to refine prognostication in prostate cancer cells, we further investigated genetic biomarkers, prostate cancer antigen 3 (PCA3), kallikrein 4 (KLK4), and KLK9 in different prostate cancer cell lines. Materials and Methods: CWR22Rv1, PC3, and DU145 cell lines were cultured until 95% confluence. The single expression of CD44 or CD133 and their combined expression were analyzed by flow cytometry, and gene expression of b-actin, PCA3, KLK4, and KLK9 was analyzed by real-time polymerase chain reaction. Results: The single expression of CD133 was less than 4% in all cell lines examined. PC3 and DU145 cells displayed a high expression of CD44 (>91%), whereas CWR22Rv1 was the only cell line that demonstrated a high co-expression of both CD44 and CD133 (>91%). In addition, PC3 and DU145 displayed low expression of PCA3, KLK4, and KLK9 when compared with their own b-actin expression. In contrast, CWR22Rva showed high expression of PCA3 and KLK4 although KLK9 expression was also low. Conclusion: Both surface and genetic biomarkers should be validated for a more accurate prognosis in prostate cancer.*

Prostate cancer is one of the most common cancers in men. Even during the COVID-19 pandemic, prostate cancer was still more widespread among deaths (1). Indeed, it has been shown that prostate cancer patients had higher susceptibility to COVID-19 infection, leading to higher mortality and hospitalization rates than other solid tumor patients (2). Therefore, the more accurate detecting methods have been critical for early cancer detection and tumor progression monitoring. Screening biomarkers has become a critical method for prostate cancer diagnosis and monitoring in order to achieve more accurate treatment (3).

Prostate cancer cell lines have been useful tools to investigate whether certain biomarkers can be applied for detecting prostate cancer. We have previously demonstrated that the expression levels of biomarkers, such as CD44 and CD133, were distinct among different prostate cancer cell lines: DU145 and PC3 were $CD44^{high}CD133^{low}$, CWR22Rv1 was $(CD44^{+}CD133^{+})^{high}$, and LNCaP displayed $CD44^{low}CD133^{low}$ characteristics (4). Therefore, not only the single and co-expression of CD44 and CD133 should be investigated when using prostate cancer cell lines, but other biomarkers should also be taken into account. In addition to surface biomarkers, the expression of some genes has been shown to be prostate cancer specific.

Prostate cancer antigen 3 (PCA3) is expressed exclusively in the prostate and is over-expressed in prostate cancer tissues (5, 6). Kallikrein (KLK) gene family includes 15 serin proteases, and comprises the largest protease family in the human genome (7). Each *KLK* gene plays a role in prostate cancer, but overall KLKs have been shown to be

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Table I. *Expression of CD44 and CD133 individually and combined in three different prostate cancer cell lines. The numbers represent average±standard deviation.*

Cell line	PC3	DU145	CWR22Rv1
Marker			
$CD44^+$ (%)	91.30±0.05	96.61±0.53	1.42±1.23
$CD133^+$ (%)	3.43±2.92	0.26±0.05	1.79±1.59
$CD44^+CD133^+$ (%)	3.67±1.70	0.81±0.55	86.43±1.33

oncogenic in prostate tumors, and to facilitate cancer invasion and metastasis (8). Therefore, the expression of PCA3, KLK4, and KLK9 was investigated in three different prostate cancer cell lines combined with the expression levels of CD44 and CD133 to understand whether they show distinct expression pattern.

Materials and Methods

Cell lines and cell culture. The PC3, DU145, and CWR22Rv1 prostate cancer cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in RPMI 1640 (Roswell Park Memorial Institute 1640, GIBCO, Thermo Fisher Scientific, Waltham, MA, USA), containing 10% Fetal bovine serum (Thermo Fisher Scientific), 1% sodium pyruvate (Hyclone, GE Healthcare, Pittsburgh, PA, USA), 1.5 g/l sodium biscarbonate (Sigma, St. Louis, MO, USA), and 1% penicillin/streptomycin (GIBCO, Thermo Fisher Scientific). Cells were seeded at the concentration of 1×10^5 cells/ml and cultured at 37˚C in a 5% CO_2 incubator. Cells were grown until 95% confluence and then processed for either flow cytometric analysis or gene expression analysis.

Flow cytometric analysis. CD44 (BD Pharmingen, San Diego, CA, USA) and CD133 (BioLegend, San Diego, CA, USA) were analyzed in this study (9, 10). The preparation of samples has been described previously (4). The expression shown in Table I was obtained from three flow cytometric experiments for each cell line, and 5,000 cells were analyzed each time.

Real-time PCR (polymerase chain reaction) analysis. Total RNA from each cell line was isolated using TRIzol™ Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. After RNA was precipitated, the pellet was washed with ethanol and resuspended in Rnase-free water. Gene expression analysis was performed using SuperScriptl™ III Platinum One-Step qRT-PCR kit (Thermo Fisher Scientific). TaqMan® probes purchased from Applied Biosystems were used to measure the target genes, and probes used in this study included b-actin (Hs99999903_m1), prostate cancer antigen 3 (PCA3, Hs01371939_g1), kallikrein related peptidase 4 (KLK4, Hs00191772_m1), and KLK9 (Hs01043981_m1). Each gene expression was analyzed six times, and the $2^{-\Delta Ct}$ method was employed to calculate the relative expression of each target gene. The results in Figure 1 show the fold change of specific genes (PCA3, KLK4, or KLK9) when compared with the endogenous gene (b-actin) in the same cell line. In addition, the $2^{-\Delta\Delta Ct}$ method was employed. The values shown in Table II represent the increased fold of the target gene in DU145 or CWR22Rv1 cells over the expression in PC3 cells.

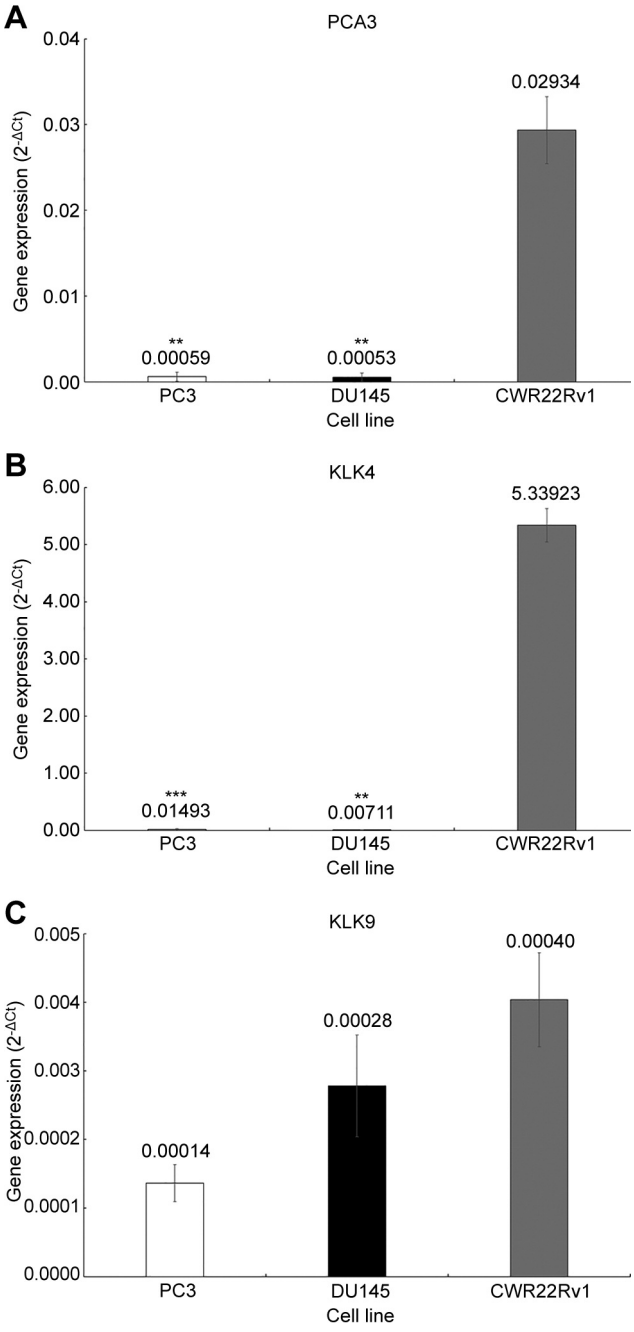

Figure 1. *The relative fold change of prostate cancer antigen 3 (PCA3) (A), kallikrein 4 (KLK4) (B), and KLK9 (C) expression after comparing b-actin expression in PC3, DU145, and CWR22Rv1 cells. **p<0.01 or ***p<0.001 when comparing gene expression in PC3 or DU145 vs. CWR22Rv1.*

Table II. *The relative expression of prostate cancer antigen 3 (PCA3), kallikrein 4 (KLK4), and KLK9 in three different prostate cancer cell lines. The values represent mean±standard deviation.*

Cell line	PC3	DU145	CWR22Rv1
$2^{-\Delta\Delta Ct}$			
PCA3	1.00±0.00	0.12±0.04	29.39±3.88**;+++
KLK4	1.00±0.00	1.23±0.88	108.83±96.47*;+
KLK9	1.00±0.00	2.16±0.97	3.31±0.18*

*$p<0.05$ or **$p<0.01$ when comparing gene expression in CWR22Rv1 *vs*. PC3. $^{+}p<0.05$ or $^{+++}p<0.001$ when comparing gene expression in CWR22Rv1 *vs*. DU145.

Table III. *The summary of biomarker expression in three different prostate cancer cell lines.*

Cell line	PC3	DU145	CWR22Rv1
Biomarkers			
CD44 and CD133	$CD44^{high}$	$CD44^{high}$	$(CD44^{+}CD133^{+})^{high}$
PCA3	Low	Low	High
KLK4	Low	Low	High
KLK9	Low	Low	Low

PCA3: Prostate cancer antigen 3; KLK4: kallikrein 4; KLK9: kallikrein 9.

Statical analysis. The differences in each gene expression were compared between PC3 and CWR22Rv1 cells, PC3 and DU145 cells, or CWR22Rv1 and DU145 cells. The 2-tailed *t*-test was assessed by Microsoft Excel, and a value of $p<0.05$ was considered significant.

Results

The single expression of CD133 was low in all three prostate cancer cell lines, and the single expression of CD44 was also low in CWR22Rv1 cells (Table I). In contrast, CD44 expression was high in both PC3 and DU145 cells and both cell lines showed over 90% expression. However, the co-expression of CD44 and CD133 was the highest in CWR22Rv1, whereas the co-expression was lower than 4% in PC3 and DU145 cells (Table I).

The expression of PCA3, KLK4, and KLK9 in each cell line is shown in Figure 1. For PC3 and DU145 cells, the expression of PC3 and KLK4 was similar to their own expression of b-actin (Figure 1A and B). In contrast, the fold change of PC3 and KLK4 on CWR22Rv1 cells was large when compared to its own b-actin expression, suggesting the expression of these two genes was high (Figure 1A-B). The expression of KLK9 was lower than 0.0004 in all three cell lines, indicating that KLK9 expression was very similar to their own b-actin expression (Figure 1C).

The $2^{-\Delta\Delta Ct}$ analysis was also utilized and the comparison is demonstrated in Table II. The expression of PCA3, KLK4, and KLK9 in DU145 cells was similar to that in PC3 cells. The relative expression of PCA3 and KLK4 in CWR22Rv1 cells was much higher compared with that in PC3 and DU145 cells. The relative expression of KLK9 in CWR22Rv1 was close to 1, but there was significant difference when compared with KLK9 expression in PC3 cells.

Discussion

The flow cytometric results in the current study are in accordance with those of our previous study: both PC3 and DU145 displayed $CD44^{high}CD133^{low}$, and CWR22Rv1 showed $(CD44^{+}CD133^{+})^{high}$. Interestingly, the expression pattern of PCA3 and KLK4 was similar to that of CD44 and CD133 expression. Both PC3 and DU145 demonstrated low expression of PCA3 and KLK4 because the expression was similar to their endogenous b-actin expression, whereas the expression of both genes was high in CWR22Rv1 (Table III). However, the expression of KLK9 was low in three prostate cancer cell lines. The results showed that the expression of biomarkers in PC3 and DU145 was similar, while CWR22Rv1 displayed different expression pattern of biomarkers.

PC3 and DU145 cells are indeed more similar, both do not express androgen receptor (AR) mRNA/protein or prostate specific antigen (PSA) mRNA/protein (11). In contrast, AR mRNA/protein and PSA mRNA can be detected in CWR22Rv1 cells but not the PSA protein (11). PCA3 and KLK4 have been suggested to be involved in prostate cancer cell proliferation and survival through AR signaling (12, 13). The doubling time of these three cell lines is approximately 40 hours (11). It is possible that cell proliferation of CWR22Rv1 is *via* AR signaling resulting in high expression of PCA3 and KLK4, whereas low expression of these two genes in PC3 and DU145 cells results from the absence of AR signaling. Blocking AR signaling in CWR22Rv1 cells might result in low expression of PCA3 and KLK4, which was observed in PC3 and DU145 cells in this study. In addition, KLK9 expression is low in all three cell lines. The role of KLK9 in prostate cancer is still unclear; it has only been shown that KLK9 expression was increased in recurrent tissue of prostate cancer patients (7). It is possible that KLK9 expression might be considered as a biomarker for analyzing tissues after treatment, but not suitable for early detection.

Biomarkers are not only used for detection of cancers pre- and post-treatment, but also considered as therapeutic targets (14). However, more studies have demonstrated that more than one biomarker needs to be considered when diagnosing and designing treatments for cancer patients (5).

Before applying any new treatments to patients, cancer cell lines are the first model used to analyze and validate them. Even for the same prostate cancer cell line, it has been shown that different subgroups demonstrate distinct properties. For example, $CD44^{high}/CD49^{high}$ PC3 cells showed better proliferative and clonogenic potential than $CD44^{low}/CD49^{low}$ PC3 cells (15). Therefore, whether a biomarker can be used as a prognostic marker requires careful investigation and verification. Testing serum levels of PSA has been a golden standard for early detection of prostate cancer, but it has also been a problem for overdiagnosis (5). If other biomarkers, such as spermidine synthase, can be used along with PSA screening, a more accurate prognosis can be achieved (16).

This study observed two groups of prostate cancer cell lines: DU145 and PC3 were $CD44^{high}CD133^{low}$; $PCA3^{low}KLK4^{low}KLK9^{low}$, while CWR22Rv1 was $(CD44^{+}CD133^{+})^{high}$; $PCA3^{high}KLK4^{high}KLK9^{low}$. The results suggested that it is critical to characterize the changes in different biomarkers in prostate cancer cells under different culture conditions (maintaining stemness or differentiation) or under different treatments, in order to understand variabilities of prostate cancer cells.

Conclusion

In this study, the distinct expression pattern of surface and genetic biomarkers in different prostate cancer cell lines was shown. Two groups of prostate cancer stem cell lines could be observed: high expression of CD44 with low expression of PCA3, KLK4, and KLK9; high expression of both CD44 and CD133 with high expression of PCA3 and KLK4. The results suggested that both surface and genetic biomarkers should be taken into account for predicting the prognosis of prostate cancer.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

All Authors contributed to the study conception and design. P.-Y. Chen performed laboratory experiments. C.-Y. Su, G.-C. Huang, and I.-C. Chen analyzed data. C.-Y. Su wrote the manuscript. G.-C. Huang, Y.-J. Chen and H.-W. Fang reviewed the data and analysis and revised the manuscript. All Authors have read and approved the final manuscript.

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