



Correlation analysis between CD133, Klk3 and grhl2 expression and tumor characteristics in prostate cancer

Jinhua Wu¹, Shulin Guo¹, Luxia Wang², Zhongxiang Liao^{1*}

¹Department of andrology, Ganzhou people's Hospital, Ganzhou, Jiangxi 341000, China

²Department of Oncology, Ganxian District People's Hospital, Jiangxi Province, Ganxian County, Ganzhou, Jiangxi 341100, China

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ABSTRACT

Prostate cancer is a common clinical disease in men. It is known that prostate cancer ranks 3rd in the incidence of malignant tumors of the male genitourinary system in China, which is able to evaluate the riskiness of life expectancy of male patients. Therefore, we investigated the expression of CD133, recombinant human kallikrein 3 (Klk3), grainy head like 2 (grhl2) in prostate cancer, and correlation with tumor characteristics in the present study. A total of 167 prostate cancer patients who underwent surgical treatment in our hospital from February 2017 to April 2021 were selected. Their cancer and adjacent tissues were resected, and CD133 was detected by double staining using immunohistochemistry, Klk3 and grhl2 were detected by RT-PCR analysis, and CD133, Klk3 were analyzed by Pearson's method in different clinical stages, Gleason grade Correlation of grhl2 with tumor characteristics. The expression of CD133, KLK3, and GRHL2 in cancer tissue was increased compared with adjacent tissue ($P < 0.05$). The expression of CD133, KLK3, and GRHL2 increased with the aggravation of the clinical stage and Gleason grade ($P < 0.05$). CD133, KLK3, and GRHL2 showed a positive correlation in prostate cancer. The Pearson method found a positive correlation between CD133, KLK3, GRHL2 and clinical stage, Gleason grade, and lymph node metastasis. In general, high CD133, Klk3, and grhl2 expression was observed in prostate cancer and increased with the disease. They presented a positive correlation in prostate cancer presence, and these three gene products correlated with tumor characteristics.

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Introduction

Prostate cancer is a common clinical disease in men. It is known that prostate cancer ranks 3rd in the incidence of malignant tumors of the male genitourinary system in China, which is able to evaluate the riskiness of life expectancy of male patients (1-3). CD133 is a unique and most common marker of stem cells, which presents an overexpression presence in a variety of tumors (4, 5). KLK3 is one of the key biological markers for the clinical detection of prostate cancer, and its detection can provide a more accurate assessment of prostate cancer (6, 7). GRHL2 is a type of tumor cell that shows aberrant expression in the prostate gland evidencing the development of prostate disease (8, 9). These three genes have been less studied in prostate cancer. In this study, they were tested with the aim of studying the expression of CD133, KLK3 and GRHL2

in prostate cancer and the correlation analysis with tumor characteristics, which can provide some clinical value for the clinical diagnosis and treatment of prostate cancer.

Materials and methods

Pneumococcal Virulence and Host Immunity

The object of the study

A total of 167 patients with prostate cancer who underwent surgery at our hospital from February 2017 to April 2021 were selected to remove the patients' cancerous and pre-cancerous tissues, with a mean age of (56.85±5.61) years, including 26 cases with PSA ≤4 ng/mL, 101 cases with PSA >4 ng/ml. The Gleason score was ≤6 in 71 cases, =7 in 84 cases and ≥8 in 12 cases. There were 96 cases with negative surgical marginal tumors and 71 cases with positive marginal tumors. All patients' general information was

*Corresponding author. E-mail: bangy14077zho@126.com
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recorded and all patients and their families were informed and signed an informed notice for this study.

Inclusion criteria included that all patients met the diagnostic criteria of the Chinese Medical Association for prostate cancer (10), all were treated surgically at our hospital, and all patients and their families were informed and signed the study information notice, which was approved by our hospital ethics committee.

Exclusion criteria included patients with co-morbid psychiatric disorders, a history of chronic alcohol or drug use, contraindications to anesthesia, and co-morbidities with other cancers.

Taking of materials

Specimens of cancerous and pre-cancerous tissues from surgically excised prostate cancer patients were prepared and fixed in 4% neutral formalin, followed by paraffin-embedded, 3- μ m-thick serial sections which were then examined by immunohistochemistry.

Immuno-histochemical staining

Serially sectioned cancer tissue was added to citrate buffer and placed in the microwave for antigen repair, followed by incubation using 3% H₂O₂, normal goat serum to seal the antibody, the addition of primary antibody and overnight at 4°C, secondary antibody added dropwise the following day, horseradish peroxidase labelling, incubation with streptavidin ovalbumin, DAB color development, re-staining with hematoxylin solution, dehydration, drying and sealing of the slices using neutral gum for CD133 detection.

Determination of immune-histochemical results

Immunohistochemistry results were determined based on the degree of cell staining and the area occupied by the cells. The intensity of staining included 0 points as no staining, 1 point as light staining, 2 points as moderate staining and 3 points as strong staining. The staining area consisted of 0 points for no staining, 1 point for <25% staining, 2 points for between 25% and 50% staining and 3 points for >50% staining. The sum of the two points >2 was recorded as positive and ≤ 2 as negative expression.

KLK3, GRHL2 assay

KLK3 and GRHL2 were assayed using RT-PCR. TRIzol reagent was added to the specimens to be tested and then allowed to stand for 10 min at 37°C.

After dissolution, 600 μ L of trichloromethane was added and stirred until the solution was milky white, and then allowed to stand for 10 min at 4°C. The supernatant was then extracted by centrifugation. The supernatant was placed in a centrifuge tube and centrifuged with isopropanol (1:1) for 15 min. 1 mL of 75% ethanol was added and total RNA was extracted. The purity and content of the extracted total RNA were tested, followed by reverse transcription to obtain cDNA. Primers were designed using Primer 5.0 software with internal reference U6. The amplification conditions were set at 94°C for the 20s, 72°C for 30s and 60°C for 30s with 35 cycles. The expression of KLK3 and GRHL2 to be assayed was calculated using the 2^{- $\Delta\Delta$ Ct} method.

Statistical analysis

Analysis was performed using SPSS 20.0 software. The measurement data were described using mean \pm standard deviation (SD), and independent samples t-test was used for comparison between groups. The statistical data were expressed as % and the χ^2 test was used for comparison between groups. The correlation between CD133, KLK3, GRHL2 and tumor stage was analyzed using Pearson's method, with P < 0.05 indicating that the difference was statistically significant.

Results and discussion

Expression analysis of CD133, KLK3 and GRHL2 in prostate cancer

As shown in Table 1, CD133, KLK3 and GRHL2 were expressed higher in cancer tissues than in paraneoplastic tissues, with statistical differences (P<0.05).

Table 1. Expression analysis of CD133, KLK3 and GRHL2 in prostate cancer ($\bar{x} \pm s$); Pre-cancerous tissues (A) and Cancerous tissue (B)

Tissue	Cases (n)	CD133	KLK3 mRNA	GRHL2 mRNA
A	167	0.53 \pm 0.04	0.29 \pm 0.03	0.61 \pm 0.25
B	167	1.53 \pm 0.25	1.59 \pm 0.21	1.69 \pm 0.33
t value		51.040	79.190	33.710
P-value		0.001	0.001	0.001

CD133 was expressed lower in paraneoplastic tissues, showed granular expression within the cytoplasm in prostate cancer tissues, and was also expressed in the nucleus in the highly expressed

tissues. Figure 1 shows CD133 expression in cancerous and paraneoplastic tissues.

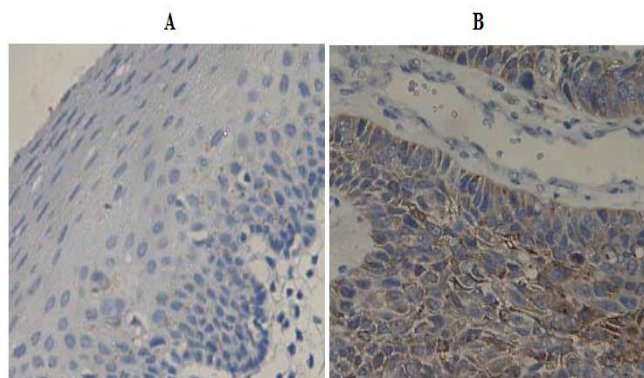


Figure 1. CD133 expression in cancerous and paraneoplastic tissues; paraneoplastic tissue (A) cancerous tissue (B).

Expression of CD133, KLK3, GRHL2 in different clinical features

As shown in Table 2, the expression of CD133, KLK3 and GRHL2 increased with the increase of Gleason's grading score, with a statistical difference ($P < 0.05$). The expression of CD133, KLK3 and GRHL2 increased with the increase of PSA content, with a statistical difference ($P < 0.05$). The expression of CD133, KLK3 and GRHL2 increased with the increase of tumour stage, with a statistical difference ($P < 0.05$). The expression of CD133, KLK3 and GRHL2 was higher in patients with lymph node metastasis than in those without lymph node metastasis, with a statistical difference ($P < 0.05$).

CD133, KLK3, GRHL2 correlation analysis

As shown in Figure 2, CD133 and KLK3 showed a positive correlation ($r=0.227$, $P=0.003$). CD133 and GRHL2 showed a positive correlation ($r=0.233$, $P=0.002$). KLK3 and GRHL2 showed a positive correlation ($r=0.293$, $P=0.001$).

Correlation analysis of CD133, KLK3, GRHL2 and tumor characteristics

As shown in Table 3, CD133, KLK3, GRHL2 and Gleason grade, tumor stage and lymph node metastasis all showed a positive correlation present.

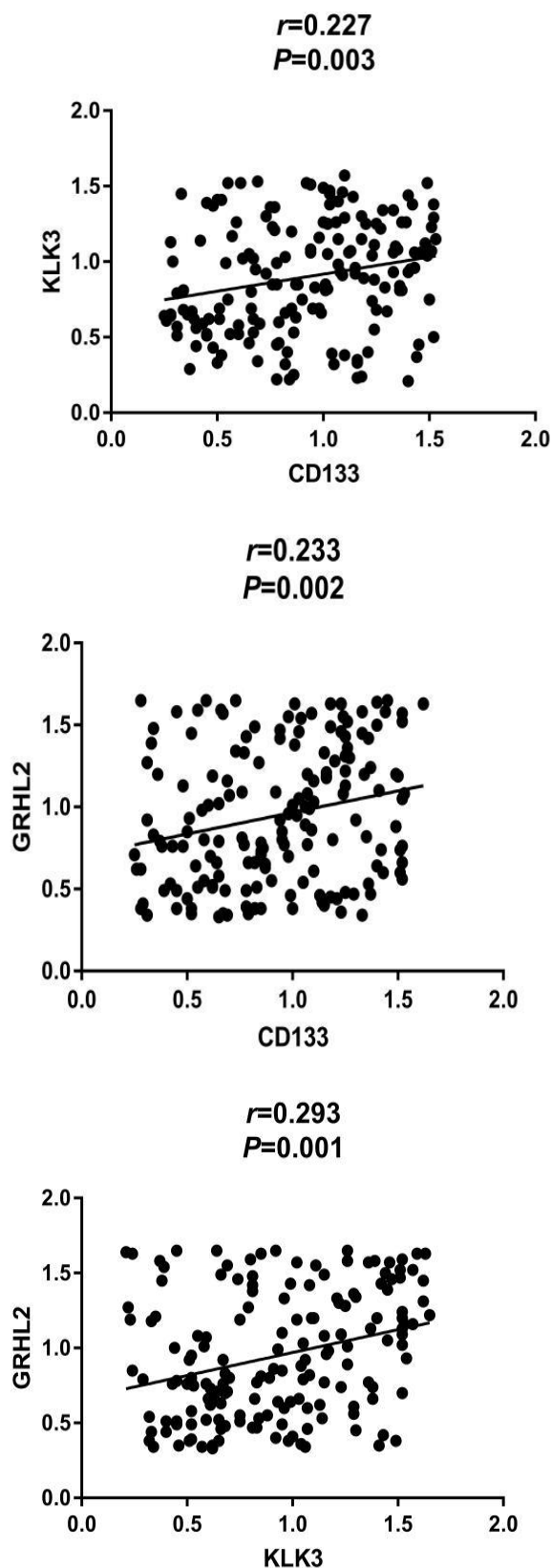


Figure 2. Correlation analysis of CD133, KLK3, GRHL2.

Table 2. Expression of CD133, KLK3, GRHL2 in different clinical features ($\bar{x} \pm s$)

Indicators		Case (n)	CD133	KLK3 mRNA	GRHL2
Gleason grading	≤6Points	71	0.53±0.01	0.35±0.02	0.52±0.16
	= 7Points	84	0.86±0.15 ^a	0.68±0.05 ^a	0.85±0.25 ^a
	≥8Points	12	1.28±0.22 ^{ab}	1.28±0.16 ^{ab}	1.33±0.25 ^{ab}
PSA	≤4ng/mL	26	0.53±0.10	0.62±0.12	0.59±0.09
	> 4 ng/mL	101	1.28±0.26 ^a	1.36±0.31 ^a	1.26±0.25 ^a
Tumour Stages	I stage	15	0.34±0.05	0.25±0.05	0.15±0.06
	II stag	69	0.95±0.16 ^a	0.62±0.11 ^a	0.29±0.10 ^a
	IIIstag	70	1.02±0.25 ^{ab}	1.06±0.29 ^{ab}	0.69±0.25 ^{ab}
	IVstag	13	1.35±0.31 ^{abc}	1.65±0.52 ^{abc}	1.62±0.86 ^{abc}
Lymph node metastass	Yes	104	1.25±0.26	1.39±0.31	1.62±0.75
	No	63	0.35±0.25 ^a	0.52±0.21 ^a	0.35±0.05 ^a

Note: ^aP < 0.05 compared with Gleason grade ≤ 6, PSA ≤ 4ng/mL, tumor stage I, and presence of lymph node metastasis; ^bP < 0.05 compared with Gleason grade = 7, tumor stage II; ^cP < 0.05 compared with tumor stage III.

Table 3. Correlation analysis of CD133, KLK3, GRHL2 and tumor characteristics

Indicators	Gleason grading		Tumour Stages		Lymph node metastasis	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
	value	value	value	value	value	value
CD133	0.689	0.001	0.956	0.001	0.846	0.001
KLK3	0.859	0.001	0.745	0.001	0.818	0.001
GRHL2	0.765	0.001	0.867	0.001	0.791	0.001

As the largest accessory gland in the male reproductive system, the prostate is divided into a central zone, a peripheral zone, and a migratory zone (11-13). Clinical studies have shown that the region of the migratory zone is a vulnerable site for prostatic hyperplasia, while the peripheral zone is the most common area for the development of prostate cancer and prostatitis (14, 15). Prostate cancer has now developed into a major global public health problem. Tumor stem cells are a population of cells present in tumor tissue that have stem cell properties, which are capable of self-renewal and multidirectional differentiation potential. The specificity of tumor stem cells and the associated cell surface markers have been a hot topic of research in recent years (16, 17). The most studied stem cell markers are CD133, CD44 and CD133 expression in different tumor tissues or in embryonic tissues, which are mainly expressed in tissue stem cell populations or tumor stem cell subpopulations (18, 19). A related animal experiment

showed that when CD133+ differentiated into endothelial cells were inoculated with lung cancer cells simultaneously with mice, the results showed that neovascularization in the tumor tissue would be higher than that in the pre-cancerous tissue. The results suggest that CD133 protein plays an important role in angiogenesis (20, 21). In this study, CD133 protein was found to be significantly higher in prostate cancer tissues than in paraneoplastic tissues, and increased with increasing tumor stage, suggesting that CD133 is closely associated with the prostate.

KLK3, a member of the serine protease kinase releasing enzyme family, is likely to be involved in prostate cancer development and metastasis. The KLK3 protein product is a protease found in seminal plasma and is clinically known as a prostate-specific antigen, which can be tested to determine prostate cancer more accurately (22, 23). Therefore, it plays an important role in the diagnosis and detection of prostate cancer. Prostate-specific antigens can serve as an important material basis for genetic susceptibility (24). It has been suggested in some clinical studies that polymorphisms in the KLK3 gene can influence the development of prostate cancer, but the clinical mechanism is not yet fully understood (25). In this study, KLK3 was found to be highly expressed in prostate cancer tissues and to increase with the progression of the disease, suggesting that KLK3

testing can provide a more accurate diagnosis of prostate cancer.

GRHL2 is a family of transcription factors that have been identified in recent years as being present in mammals. The GRH family is first identified in *Drosophila* where it is involved in the regulation of embryonic and neural development, epidermal morphogenesis and repair of a variety of physiological functions in *Drosophila*. The GRH family is capable of behaving as activators and inhibitors. The role of GRHL2 in tumor development has received considerable attention in recent years (26, 27). Clinical studies have shown that GRHL2 acts differently in many different types of tumors, promoting tumor growth and acting as a suppressor to inhibit tumor growth (28). For example, GRHL2 has a suppressive effect in the tight junction low expression subgroup and in the mesenchymal subgroup in breast cancer, but a pro-carcinogenic effect in other subtypes of breast cancer (29, 30). In this study, GRHL2 expression was found to be highly expressed in the pre-cancerous tissues of prostate cancer patients and increased with the stage and Gleason grade of the disease, suggesting that GRHL2 is closely related to prostate cancer.

In this study, CD133, KLK3 and GRHL2 were analyzed and their expression in cancer tissues showed a positive correlation. The correlation between the three and Gleason's classification, tumor stage and lymph node metastasis also showed a positive correlation, indicating that the three correlate with tumor characteristics.

In summary, CD133, KLK3 and GRHL2 showed high expression presence in prostate cancer and all three showed positive correlation presence in prostate cancer and with tumor characteristics.

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None.

Interest conflict

None.

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