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STEAP1 as a New Diagnostic Marker Candidate for Prostate Cancer

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ABSTRACT

Objective: Prostate cancer is the second most common cancer among men in the world. Although the diagnosis rate through PSA has increased, PSA also showed false positive even in non-prostate cancer patient. Therefore, it needs to provide more specific diagnostic markers. STEAP1 is involved in cell to cell communication. It is also highly expressed specifically in prostate cancer. Therefore, by evaluating the expression level of STEAP1 in exosomes, we intend to evaluate its potential use as a diagnostic marker.

Design: In order to test the expression level of STEAP1 in the prostate cancer cell line, the gastric cancer cell line and keratinocyte, RT-PCR was initially used. To check whether STEAP1 is present in the exosome, the protein expression level was checked using western blot and its role in cell migration was investigated.

Result: STEAP1 exhibited a high expression level only in prostate cell lines but not in other cancer cell and control cell lines by qRT-PCR. The expression level of STEAP1 derived from exosome was also found to be 3-4 times higher in cancer cell lines than in normal prostate cell lines in Western blot analysis. Migration and invasion rate of AGS cells increased when exosome containing STEAP1 was treated.

Conclusion: Even though mRNA expression of STEAP1 is high in prostate cell lines, exosomal STEAP1 protein is only detected in the prostate cancer cell lines. Therefore, exosome-derived STEAP1 can be used as a diagnostic marker for prostate cancer. In addition, exosome-derived STEAP1 may increase metastasis of other cancers, so it should be a new target for the cancer metastasis.

Key Words: Prostate cancer; STEAP 1; Prognosis; Diagnostic Marker; Cancer cell lines

ABBREVIATION

Prostate cancer (PCa)

Prostate-specific antigen (PSA)

Six-transmembrane epithelial antigen of the prostate 1 (STEAP1)

INTRODUCTION

Prostate Cancer (PCa) is a common cause of cancer death and one of the most commonly diagnosed tumors in Western countries [1]. In 2012, about 1.1 million new cases were diagnosed and 307,000 people died worldwide [2]. Prostate specific antigen (PSA); human kallikrein 3 (hK3) that encoded by the KLK3 gene is a glandular kallikrein with abundant expression in the prostate. PSA has been mainly used for the diagnosis of prostate cancer. When it enforced first prostate cancer diagnosis test with PSA in 1986-1991, the incidence rate increased dramatically [3]. However, in many subsequent prostate biopsies, elevated PSA levels are not correlated with the presence of cancer. Positive results are also seen in patients with benign prostatic hyperplasia and prostatitis. Therefore, PSA is positive in only about 25% of patients in the range of 2-10 μg/L. Also, since PSA is detected in patients with enlarged prostate volume [4]. It shows low specificity and leads to unnecessary overdiagnosis.

However, studies show a 13%-20% increase in death from prostate cancer without the use of PSA [2]. Therefore, there is a need for a new biomarker that can diagnose prostate cancer

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more accurately than PSA.

Exosomes are nano-sized vesicles secreted by many cells. Exosomes are surrounded by a lipid bilayer and transport various biomolecules when taken up by other cells. Therefore, it is recognized as an essential medium of cell to cell communication. Intercellular communication is a key function of tumor progression and metastasis. Because exosomes transport and deliver bioactive molecules, there is much interest in whether they can control metastasis. Cancer exosomes express various proteins such as oncogenic proteins, integrins, and signaling molecules [5]. Therefore, it needs to study the potential of cancer exosome proteins as diagnostic markers.

STEAP is known to be highly expressed in prostate cancer. It is also known to be expressed in the pancreas, ovaries, gastrointestinal tract, cervix, and bladder. However, its expression level is low in other cancers, making it a very popular biomarker and immunotherapeutic target in prostate cancer [6]. In fact, when STEAP1 expression was suppressed in LNCaP, one of the prostate cancer cell lines that express a lot of STEAP1, apoptosis and cell proliferation was suppressed [7]. STEAP1 is mainly located in the plasma membrane of epithelial cells. It is also dispersed in the cytoplasm and may act as a transporter protein at the cell-cell junction, which may be involved in intercellular communication. Therefore, it was expected that STEAP1 would be included in exosome, and there is a possibility as a diagnostic marker.

METHODS AND MATERIALS

Quantitative Real-Time PCR

The experimental method is same with the previous experimental method [8]. Total RNA was isolated using TRIzol reagent (Life Technology, Thermo Fisher Scientific, USA), and 1 μg of total RNA was used to synthesize the cDNA using Thermo reverse transcriptase (NanoHelix, Korea). 1 μg of cDNA used as template for quantitative real time PCR using QGreen 2X SybrGreen qPCR Master Mix (CellSafe, Korea). GAPDH was used as a quantitative control. Gene expression was calculated by delta Cq formula. All of primer sequences was shown in **Table 1**.

Table 1. Primer sequences for quantitative-Real time PCR

Gene	5'-3'	Primer sequence	Annealing temp. (C°)	Primer
STEAP1	Forward	TTC AGC ACA CAC AGG AAC TC	56.0	In this study
	Reverse	CAA TGC CAA GAG AGT GAT GG	54.5	
GAPDH	Forward	GTG AAG GTC GGA GTC AAC G	57.1	[8]
	Reverse	TGA GGT CAA TGA AGG GGT C	55.3	

Cell Sample for Western Blot

All cells were grown until they reached 60%, replaced with serum free media to obtain exosomes. The cells were lysed using radioimmunoprecipitation assay buffer (RIPA) lysis buffer with 150 mM sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 mM Tris (pH 8.0), and complete protease inhibitor cocktail (BIOMAX, Korea). Protein was quantified by Bradford (Sigma-Aldrich, USA) assay. Bradford assay was performed in the same way as before [8].

Exosome Isolation

Total Exosome Isolate (Invitrogen™, #4478359) was used to isolate exosome based on manufacturer's instruction [9]. In order to quantify and compare the amount of exosome production regardless of the type of cell, all of the medium (10 ml) was centrifuged at 2000 g for 30 minutes, and then 8 ml of supernatant were transferred. After, total exosome was isolated. The-isolated exosomes were stored at -78°C before use.

Western Blot Analysis

The experiment was done with the same way as previously conducted. Proteins were separated from the same amount (20 μg) of the prepared sample using 10% acrylamide SDS-PAGE gel. For the cell pellet, cells grown in a medium from which exosomes were separated were used. Primary antibody (STEAP1, #ab207914) and secondary antibody (2nd anti-mouse, #ab6728) were used.

Transmembrane Assay

The experiment was performed by slightly modifying the protocol of transmembrane assay [10,11]. Seed cells in the upper chamber with 1.5 ml of serum-free medium. 24 hours later, the exosome treated on the upper chamber, and the 2 ml of medium containing FBS is added in the lower chamber. After 24 hours, to remove the remaining cells, wash twice with PBS. Cells were stained with 0.5% crystal violet and washed 3 times with PBS. Stained cells were observed under a microscope and counted.

Statistics

The experiments were repeated at least three times independently. Results are expressed with ± standard deviation. Using a one-way ANOVA, statistically significant differences were analyzed. When only two groups were compared, the student's t-test was used (*:P<0.05, **:P<0.01).

RESULTS

Candidate Identification by General Schemes

From extracellular vesicle database; Vesiclepedia the 7145 gene encoding exosomal protein detected in exosomes of prostate, kidney, lung, breast and colorectal cancer were choose. Of the 7145 genes, 180 genes were selected based on the frequency of occurrence in the cancer derived exosome. Among them, 168 expressions were tested, and 15 candidates were found. Except for 12 genes previously reported, STEAP1, which is known to be widely expressed in prostate cancer, was select-

ed from among the remaining genes, and further experiments were carried out.

Exosomal STEAP1 Expression in Cancer Cell Lines

The expression level of STEAP1 was tested in various cell lines including prostate cell lines. As it is known, LNCaP had the highest expression level, but expression at the RNA level was hardly observed in other cell lines except for prostate cell lines (Figure 1). Western blot was performed with a cell pellet to check the protein expression level in the prostate cell line. When the divided value compared to GAPDH was quantified compared to RWPE-1, a normal prostate cancer cell line, the prostate cancer cell lines DU145, PC3, and LNCaP clone FGC showed similar expression levels of 0.6, 1.1, and 0.8, respectively (Figure 2).

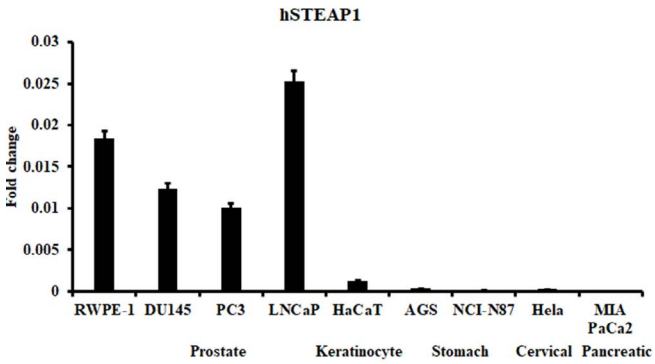

Figure 1: The expression of STEAP1 showed high in prostate cell lines A) RNA expression level of STEAP1 showed high in prostate cell lines. To check the RNA expression level of STEAP1 in several cell lines, cDNA was synthesized and qRT-PCR was performed. Expression was confirmed in all prostate cell lines, but not in other cell line

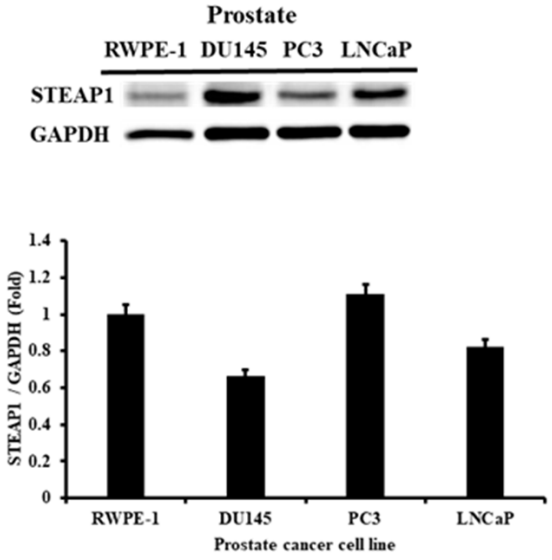

Figure 2: Protein expression level of STEAP1 in cell was not differ. Western blot was performed to confirm the expression of STEAP1 in each of the prostate cell lines. The band was quantified using image J.

Exosomal protein was also checked for expression in all prostate cell lines. As a result of confirming the expression level of exosomal STEAP1, the expression level of STEAP1 in RWPE-1 was almost absent, and the expression of the protein amount was 16, 14, and 22 times higher in the prostate cancer cell lines DU145, PC3, and LNCaP than in RWPE-1, respectively (Figure 3).

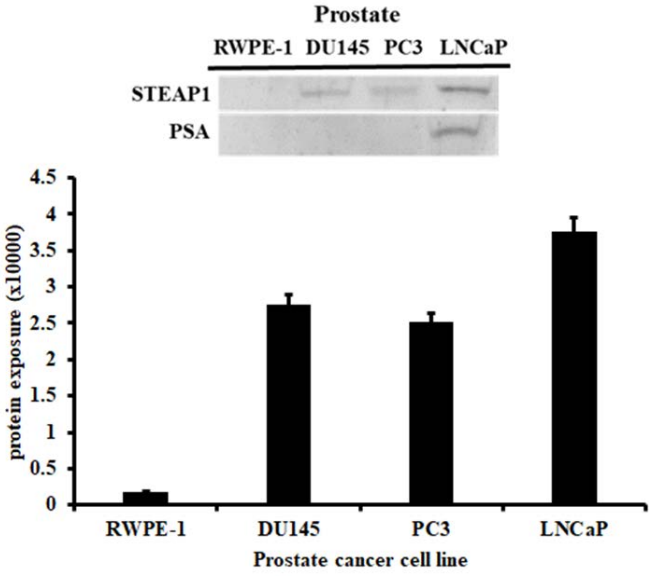

Figure 3: Protein expression level of exosomal STEAP1 in prostate cell lines is high compare with normal prostate cellThe protein expression level of exosome-derived STEAP1 was tested. The medium was replaced with a medium without FBS and the medium was obtained after 24 hours to isolate the exosomes. The protein expression level was tested by western blot. Each band was quantified through image J. The graph shows the comparative expression level of STEAP1 compared to GAPDH.

Exosomal STEAP1 Induce the Cancer Migration

Using the stomach cancer cell line AGS with a low STEAP1 expression level, it was checked whether STEAP1 increased cancer cell metastasis. In AGS cells treated with DU145 exosomes, which expressed a lot of STEAP1 and had a similar doubling time with AGS, the amount of metastasis was increased in a concentration dependent manner compared to the untreated control. When the metastasis of untreated control was considered as 100%, 200 ul of exosomes treated cancer cells showed about 59% increased metastasis, and 400 ul of exosomes treated cancer cells showed about 243% increased metastasis (Figures 4 and 5).

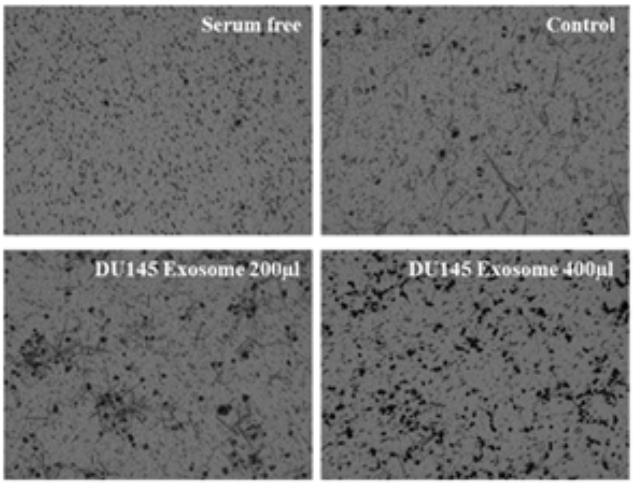

Figure 4: Exosomal STEAP1 induced the cancer cell metastasis. AGS, a gastric cancer cell line lacking the expression level of STEAP1, was

used in the experiment. STEAP1 treated DU145 exosomes and the number of cells was checked using crystal violet staining

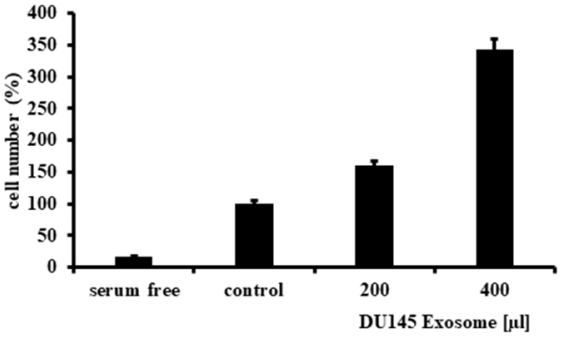

Figure 5: Exosomal STEAP1 induced the cancer cell metastasis. Graph represents the DU145 Exosome vs Cell Membrane

DISCUSSION

PSA is widely used in the diagnosis of prostate cancer. However, PSA only detects LNCaP among prostate cancer cell lines. However, even in the same cancer, the secreted protein is slightly different depending on the type of cancer [12]. Therefore, there is a need for a new biomarker that can comprehensively diagnose prostate cancer, not just LNCaP. After finding candidates in the extracellular vesicle database, genes were screened. As a result of screening for several cancer cell lines, STEAP1 was not expressed in other cancer cell lines, but showed a high expression level only in prostate cell lines. Therefore, we thought that the expression of STEAP1 was specifically high in prostate cell lines, so it was selected as a candidate. As a result of confirming the expression level of STEAP1 in the entire cell by western blot, it showed almost the same amount. So, it was checked how much of STEAP1 were released into the exosomes, and found that only STEAP1 of prostate cancer is released as exsomes.

The fact that STEAP1 was identified only in the exosomes of cancer cell lines suggests that STEAP1 helps cancer growth. Thus, it was thought that it may be related to cancer metastasis. STEAP1 is known to promote cancer cell metastasis through the JAK2/STAT3 signaling pathway [13]. AGS showed very low expression level of STEAP1. To determine whether STEAP1 affects cancer metastasis, AGS was treated with DU145 exosomes. As a result, the metastasis of AGS was increased. Thus, we suggest that reducing the expression level of exosomal STEAP1 could reduce prostate cancer metastasis. However, further studies including whether only depressed amount of exosomal STEAP1 not cellular STEAP1 should induce the cancer metastasis or not, and in vivo mice assay must be followed to show the usage of STEAP1 inhibitor as a anti metastasis candidate [6,14,15].

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CONFLICTS OF INTEREST

The authors have no conflict of interest to report.

ETHICAL APPROVAL

Not required

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