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Communication

Investigating the role of caveolin-2 in prostate cancer cell line

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Abstract: Prostate cancer is a worldwide problem. While the role of caveolin-1 has been extensively studied, little is known about the role of caveolin-2 (CAV2) in prostate cancer. Up-regulation of CAV2 in androgen independent PC3 cells compared to normal prostate cell line and androgen dependent prostate cancer cell lines has been observed. Recent studies suggest that up-regulation of CAV2 plays an important role in androgen independent prostate cancer. This study investigates whether *CAV2* is important in mediating the aggressive phenotypes seen in androgen independent prostate cancer cells. The androgen independent prostate cancer cell line, PC3 was used that has been shown to express CAV2, and *CAV2* knock down was performed using siRNA system. Changes to cell number, migration and invasion were assessed after knocking down *CAV2*. Our results showed that down-regulating CAV2 resulted in reduced cell numbers, migration and invasion in PC3 cells. This preliminary study suggests that *CAV2* may act to promote malignant behavior in an androgen independent prostate cancer cell line. Further studies are required to fully elucidate the role of *CAV2* in androgen independent prostate cancer.

Keywords: caveolae; caveolin-2; migration; invasion

1. Introduction

Caveolae are small, "omega-shaped" invaginations at the plasma membrane that have been shown to be involved in signal transduction, mechanosensing and vesicle trafficking [1,2]. Within caveola, there are caveola-associated molecules, including; caveolin-1 (CAV1), caveolin-2 (CAV2) and caveolin-3 (only expressed in muscle cells) [2]. Caveolin-2 (CAV2) is a 22kDa protein that is present abundantly in white adipose tissue and its expression is induced during the process of adipocyte differentiation [3]. Although reports suggest that the expression of CAV2 is not essential in

caveola formation, co-expression of this molecule with CAV1 results in more caveolae being formed with better invaginations, suggesting that CAV2 plays a supporting role in this process [4,5]. CAV2 is reported to be expressed concurrently with CAV1 and is able to form oligomers with CAV1 in adipocytes, fibroblasts and endothelial cells through the process of hetero-oligomerization [6]. In addition, Rybin et al. reported that CAV2 is able to interact with CAV3 in cardiac muscle cells [7].

With the successful generation of *CAV2* knock out mice, the importance of this gene in pathophysiological processes has been characterized. *CAV2* knock out mice demonstrate changes in the lung, which include increased numbers of endothelial cells, thickening of alveolar septa and hypercellularity of lung parenchyma [5]. However, *CAV2* null mice do not show any changes in the number of caveolae [5,8].

The expression of CAV2 has also been reported to be dysregulated in several cancers. Immunohistochemical evaluation of CAV2 expression in clinical specimens suggests that CAV2 has a tumor promoter role in breast cancer because CAV2 is significantly associated with higher tumor grade, larger tumor size and decrease in overall survival [9,10]. Apart from breast cancer, there are reports that the expression of CAV2 is associated with tumor grade in bladder cancer and decreased survival rate in esophageal squamous cell carcinoma [11,12].

In prostate cancer, Gould et al. reported that the expression of CAV2 is reduced in androgen dependent LNCaP cells but present in androgen independent PC3 prostate cancer cell line. The aim of this study is to understand the significance of CAV2 expression in an androgen independent prostate cancer cell line and whether down-regulating *CAV2* reduces the aggressive phenotypes of these cells.

2. Materials and Methods

2.1. Cell culture

Androgen independent prostate cancer cell line, PC3 (ATCC: CRL-1435) were grown in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA, USA). Cells were grown in a humidified incubator at 37 °C with 5% CO_2.

2.2. Gene knock-down using CAV2 siRNA

Pooled small interfering RNA (siRNA) that targets human *CAV2* was purchased from Santa Cruz (Santa Cruz Biotechnologies, California, USA). For a control, scrambled sequence of siRNA (control siRNA) that was known to not cause specific degradation of mRNA, was purchased from Santa Cruz (Santa Cruz Biotechnologies, California, USA). Cells were transfected as described by manufacturer's protocol. Briefly, 2.5×10^4 cells or 8.0×10^4 were seeded in 24 or 6 well plate. Three microliter of siRNA Transfection Reagent (Santa Cruz Biotechnologies, California, USA) and a final concentration of 120 nM of siRNA were added together into each well in a final volume of 200 μL. Cells were returned to the humidified incubator at 37 °C with 5% CO_2 for 8 hours and thereafter 200 μL of complete medium was added to each respective well. Cells were cultured for 24 hours. The following day, culture medium from each well was removed and replaced with fresh complete medium. Cells were cultured for another 48 hours and were harvested for further experiments.

2.3. Western blot

Seventy-two hours post transfection, cells were harvested for Western blot analysis. Briefly, a total of 5.0×10^4 cells suspended in Complete® Protease Cocktail Inhibitor (Roche Diagnostics, Basel, Switzerland) was loaded to each well and electrophoresed at 100 V for 90 mins and transferred in ice cold buffer at 200 mA for 90 mins. Membrane was then blocked for an hour with Odyssey Blocking Solution (LI-COR Biosciences, Lincoln, Nebraska, USA) over a shaker and thereafter, incubated with CAV2 antibody (1:2000) (Cell Signaling Technology®, Danvers, MA, USA) along with GAPDH antibody (1:10,000) (Abcam, Cambridge, UK) overnight at 4 °C diluted with Odyssey Blocking Solution. The following day, a secondary antibody was added at a dilution of 1:10,000 (or 1:20,000 for GAPDH) (LI-COR Biosciences, Lincoln, Nebraska, USA) for two hours in room temperature protected from light. The membrane was then washed with TBS and TBS-T before being dried and scanned with Odyssey Infrared Imager (LI-COR Biosciences, Lincoln, Nebraska, USA). Quantification of protein expression was performed using Odyssey Application Software Version 3.0 (LI-COR Biosciences, Lincoln, Nebraska, USA). The result was expressed as optical density of the protein expression relative to the housekeeping protein.

2.4. Proliferation assay

Proliferation assays were performed using the CellTiter 96 Aqueous Non-Radioactive cell proliferation kit (Promega, Madison, USA). Briefly, 72 hours post transfection, cells were incubated with the assay reagent and the absorbance was measured at 490nm wavelength using Synergy 2 Multi-Mode Microplate Reader (Biotek, Winooski, USA).

2.5. Migration assay

Migration assays were carried out with the use of Transwell inserts (Corning, NY, USA). Cells were harvested 72 hours post transfection and were re-suspended in serum free medium before being re-seeded (8.0×10^4) into the pre-hydrated migration insert and was left migrated for 48 hours in the presence of medium supplemented with 20% of FBS as chemo-attractant. Migration inserts were washed with 1× PBS and fixed with 4% paraformaldehyde for 15 mins. Then, inserts were washed with 1× PBS and left to air dry before being stained with 0.5% (w/v) aqueous crystal violet for 30 mins. Tap water was used to wash the inserts to remove remaining crystal violet in the inserts. With the use of a pre-wet cotton swab, cells that did not migrate through the polycarbonate membrane were removed from the upper surface of the inserts. Inserts were then visualized under a stereo microscope (Nikon SMZ 1500). Three random images per insert were taken with the use of a digital camera and its software (Nikon DXM1200F). The number of migrated cells in each image was counted.

2.6. Invasion assay

BD Biocoat Matrigel invasion chambers were purchased from BD Biosciences (BD Biosciences, CA, USA). Cells were harvested 72 hours post transfection, re-suspended in serum free medium before reseeded (8.0×10^4) into the invasion chamber and incubated for 48 hours in the presence of

medium supplemented with 20% of FBS as chemo-attractant. Forty-eight hours later, invasion inserts were fixed and stained as described in migration assay.

2.7. Statistical analyses

Statistical analyses were performed with Graph Pad Prism 6.0 software (La Jolla, California, USA). Data are presented as mean ± SE. Three replicates of sample were used per experiment and the mean of the three replicates was recorded. Each experiment was then repeated three times, where the final sample size (n = 3) denotes the mean results from 3 independent experiments. Statistical significance was achieved with a p-value < 0.05.

3. Results and Discussion

Recently, a study examined the plasma level of CAV2 in castrate resistance prostate cancer (CRPC) and showed that CAV2 was significantly up-regulated in the plasma samples compared to non-CRPC patients [13]. However, there was no association with clinico-pathological parameters such as Gleason grade and tumor stage [13]. The authors also showed that the expression of CAV2 and CAV1 was significantly up-regulated in androgen independent PC3 cells compared to androgen dependent LNCaP cells [13], confirming the earlier findings of Gould et al. [14]. This may suggest that CAV2 may act as a tumor promoter in CRPC.

Therefore, we postulated that knocking down *CAV2* in PC3 cells would reduce the aggressive phenotype of the cells. We used androgen independent PC3 cells as a model of our study and PC3 cells were exposed to *CAV2* siRNA for 72 hours before harvested for western analysis for CAV2 expression. Western blot demonstrated successful reduction of CAV2 protein level (Student's t-test $p < 0.001$) (Figure 1).

Knocking down *CAV2* significantly reduced the number of PC3 cells (Student's t-test $p < 0.001$) (Figure 2A). There was a significant decrease in cell migration compared to controls (Figure 2B) (Student's t-test $^*p < 0.05$). Decreased cell invasion was also noted after knocking down *CAV2* in PC3 cells (Student's t-test $p < 0.01$) (Figure 2C). Therefore, this study suggests that *CAV2* may play a role in mediating the aggressive phenotypes in CRPC.

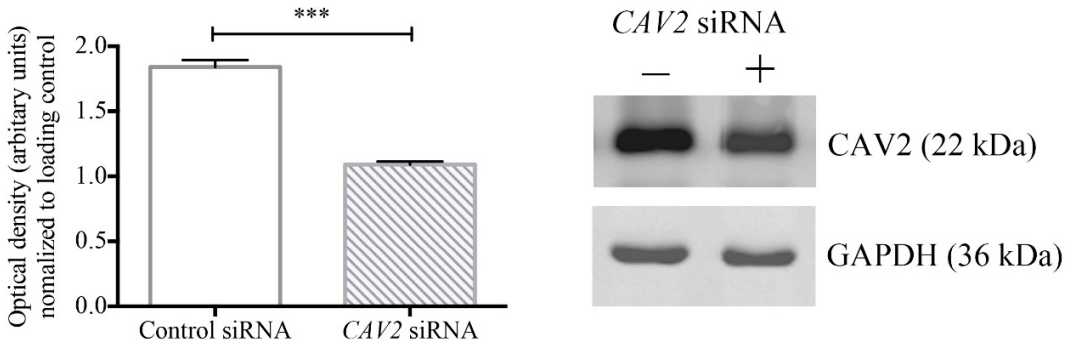

Figure 1. Western blot analysis of CAV2 expression after *CAV2* siRNA treatment. Data represent mean ± SE, Student's t-test $^{***}p < 0.001$, n = 3.

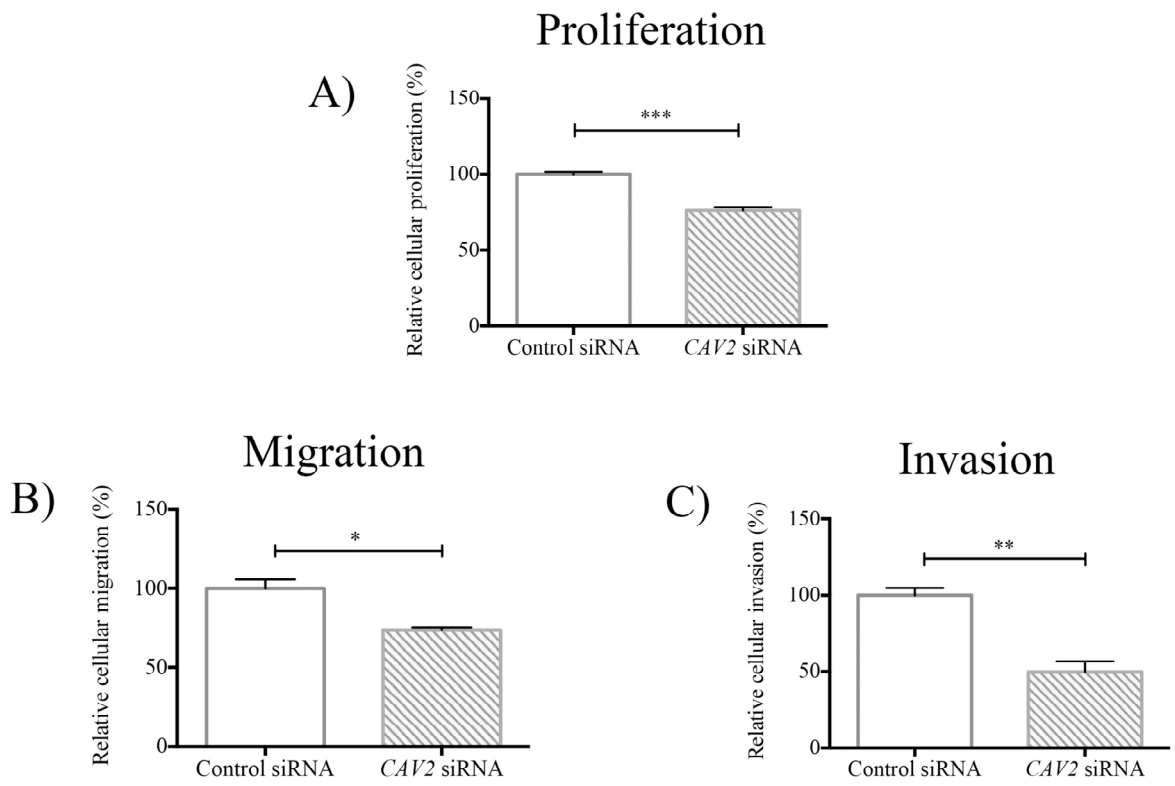

Figure 2. (A) Reduction in cell number was noted after knocking down *CAV2*. (B) Knocking down *CAV2* significantly decreased migration capacity of PC3 cells. (C) *CAV2* knock down significantly decreased the invasion of PC3 cells. Data represent mean ± SE, Student's *t*-test *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, $n = 3$.

Nevertheless, there are several limitations to this study. This study only looked at one time point and use a single siRNA concentration to knock down *CAV2*. Also, only a single cell line is used to examine the effect of knocking down *CAV2*. Furthermore, this study only used a pool of three *CAV2* siRNAs for the experiments. While siRNA pools have several advantages over individual siRNA duplexes including; greater phenotypic penetrance and a greater likelihood to generate loss of function phenotypes [15], in the future, further studies using another sequence of siRNA are required to validate the specificity of the effects observed in this study. Subsequently, knocking down *CAV2* in other prostate cancer cell lines and over-expressing *CAV2* in prostate cancer cells with low endogenous *CAV2* will further support our current findings. Collectively, our current data should be interpreted with care until it is supported by future studies as described above.

Apart from prostate cancer, recent reports also suggest a role for CAV2 in several other cancers. In breast cancer, the expression of CAV2 is associated with a decrease in overall survival of patients and it has been postulated that CAV2 has a tumor promoter role in breast carcinogenesis [9]. Further, up-regulation of *CAV2* expression was observed in inflammatory disease of the breast and triple negative breast cancer [10,16]. CAV2 is also positively associated with the tumor grade of urothelial carcinoma of the bladder [11]. Similarly, in patients with esophageal squamous cell carcinoma CAV2 immunopositivity was associated with a decreased survival rate compared to patients who were negative for CAV2 [12]. It has been shown that the loss of microRNA-218 leads to an increase in

aggressive phenotypes (migration and invasion) of renal cell carcinoma by directly targeting *CAV2* [17]. Taken together, it is tempting to suggest that *CAV2* acts as a tumor promoter in these cancers. Nevertheless, further experiments are required before the prognostic and therapeutic value of CAV2 can be fully elucidated.

4. Conclusion

Our preliminary findings suggest that up-regulation of *CAV2* may play a role in mediating the aggressive phenotypes seen in androgen independent prostate cancer. However, more studies are required to fully understand the biology of *CAV2* in androgen independent prostate cancer.

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Conflict of interests

The authors declare no conflict of interests in this publication.

References

1. Anderson RG (1998) The caveolae membrane system. *Annu Rev Biochem* 67: 199-225.
2. Parat MO (2009) The biology of caveolae: achievements and perspectives. *Int Rev Cell Mol Biol* 273: 117-162.
3. Scherer PE, Okamoto T, Chun M, et al. (1996) Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. *Proc Natl Acad Sci U S A* 93: 131-135.
4. Li S, Galbiati F, Volonte D, et al. (1998) Mutational analysis of caveolin-induced vesicle formation. Expression of caveolin-1 recruits caveolin-2 to caveolae membranes. *FEBS Lett* 434: 127-134.
5. Razani B, Wang XB, Engelman JA, et al. (2002) Caveolin-2-deficient mice show evidence of severe pulmonary dysfunction without disruption of caveolae. *Mol Cell Biol* 22: 2329-2344.
6. Das K, Lewis RY, Scherer PE, et al. (1999) The membrane-spanning domains of caveolins-1 and -2 mediate the formation of caveolin hetero-oligomers. Implications for the assembly of caveolae membranes in vivo. *J Biol Chem* 274: 18721-18728.
7. Rybin VO, Grabham PW, Elouardighi H, et al. (2003) Caveolae-associated proteins in cardiomyocytes: caveolin-2 expression and interactions with caveolin-3. *Am J Physiol Heart Circ Physiol* 285: H325-332.
8. Razani B, Woodman SE, Lisanti MP (2002) Caveolae: from cell biology to animal physiology. *Pharmacol Rev* 54: 431-467.
9. Savage K, Leung S, Todd SK, et al. (2008) Distribution and significance of caveolin 2 expression in normal breast and invasive breast cancer: an immunofluorescence and immunohistochemical analysis. *Breast Cancer Res Treat* 110: 245-256.

10. Elsheikh SE, Green AR, Rakha EA, et al. (2008) Caveolin 1 and Caveolin 2 are associated with breast cancer basal-like and triple-negative immunophenotype. *Br J Cancer* 99: 327-334.
11. Fong A, Garcia E, Gwynn L, et al. (2003) Expression of caveolin-1 and caveolin-2 in urothelial carcinoma of the urinary bladder correlates with tumor grade and squamous differentiation. *Am J Clin Pathol* 120: 93-100.
12. Ando T, Ishiguro H, Kimura M, et al. (2007) The overexpression of caveolin-1 and caveolin-2 correlates with a poor prognosis and tumor progression in esophageal squamous cell carcinoma. *Oncol Rep* 18: 601-609.
13. Sugie S, Mukai S, Yamasaki K, et al. (2015) Significant Association of Caveolin-1 and Caveolin-2 with Prostate Cancer Progression. *Cancer Genomics Proteomics* 12: 391-396.
14. Gould ML, Williams G, Nicholson HD (2010) Changes in caveolae, caveolin, and polymerase 1 and transcript release factor (PTRF) expression in prostate cancer progression. *Prostate* 70: 1609-1621.
15. Parsons BD, Schindler A, Evans DH, et al. (2009) A direct phenotypic comparison of siRNA pools and multiple individual duplexes in a functional assay. *PLoS One* 4: e8471.
16. Van den Eynden GG, Van Laere SJ, Van der Auwera I, et al. (2006) Overexpression of caveolin-1 and -2 in cell lines and in human samples of inflammatory breast cancer. *Breast Cancer Res Treat* 95: 219-228.
17. Yamasaki T, Seki N, Yoshino H, et al. (2013) MicroRNA-218 Inhibits Cell Migration and Invasion in Renal Cell Carcinoma Through Targeting Caveolin-2 Involved in Focal Adhesion Pathway. *J Urol* 190: 1059-1068.

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