SUPPLEMENTAL INFORMATION AND DATA FOR

Addiction to Protein Kinase C\textsubscript{\textalpha} due to PRKCI Gene Amplification can be exploited for an Aptamer-Based Targeted Therapy in Ovarian Cancer

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SUPPLEMENTARY INFORMATION AND DATA

Oligonucleotide Sequences for Aptamers

The siRNA sequence for Scramble Control (25/27mer) is the following:

Forward: 5’ CUUCCUCUCUUUCUCUCCCUUGUGA 3’
Reverse: 3’ UCACAAGGGAGAGAAAGAGGAAGGA 5’

The siRNA sequence against PKCɩ (25/27mer) is the following:

Forward: 5’ UUAUGAGCUAAAACAAGGAUUCUGA 3’
Reverse: 3’ GAAAAUCUCGAAAUGUUCUCUAAGACU 5’

Partially Single-Stranded Oligo Template Design Ordering Info for Aptamers:

T7 Promoter: 5’ AATT TAATACGACTC AACTATA TAG 3’

PKCɩ Oligo 1:
5’ ttcaagatcccttgtttagctcatattacgaccggtaacccagtgcctgcTATAGTGAGTCGT ATTA AATT 3’

PKCɩ Oligo 2:
5’ ctttatgagctaaacaggattctgaattacgaccggtaacccagtgcctgcTATAGTGAGTCGT ATTA AATT 3’

Partially Single-Stranded Oligo Template Design Ordering Info:

Scramble Oligo 1:
5’ tcacaaggagagaagagaggaagttacgaccggtaacccagtgcctgcTATAGTGAGTCGT ATTA AATT 3’

Scramble Oligo 2:
5’ agtgttccctcttctctctctctctccctacaagcgggtaacccagtgcctgcTATAGTGAGTCGT ATTA AATT 3’

To anneal synthetic oligonucleotides to make a transcription template, it is only necessary that the promoter sequence of a template be double-stranded. The T7 Promoter sequence was annealed to form a double-stranded promoter using the oligonucleotides listed above. The oligonucleotides were suspended in TES (10mM Tris-HCl, pH 8, 1mM EDTA, 0.1M NaCl) and mixed in equimolar
amounts to a final concentration of ~10-50µM of each oligonucleotide. The mixture was brought to 95°C in a heat block for a few minutes and then allowed to cool to room temperature. Afterwards, these annealed, partially single-stranded templates generated were then used in the in vitro transcription kits (Promega). Aptamers were generated using RNA generated from 2 sequences, hence Oligo 1 and Oligo 2 listed above.

Visualization for Aptamers

The Forna package is an RNA secondary structure visualization tool that was used to edit and display the RNA-based structures of the aptamers generated in this study (http://rna.tbi.univie.ac.at/forna/).

Aptamer Synthesis

Control and PKCiota aptamers were individually synthesized by in vitro transcription (Promega) with phage promoter-containing synthetic oligonucleotides as templates (IDT). The oligonucleotides were PAGE or HPLC purified to remove partial sequences. The T7 RNA polymerase promoter is underlined and the EpCAM aptamer is bolded.

Forward Primer for EpCAM (28nt):

5’ TAATACGACTCATAAGCGACTGGTTA 3’

ssDNA of EpCAM aptamer (62nt):

5’ TAATACGACTCATAAGCGACTGGTTACCCGGTCGT 3’

Each aptamer is composed of two RNA structures that were generated in separate in vitro transcription reactions but then were annealed together in a 1:1 molar ratio. Normal purine and pyrimidines were added to the mixture but 2’fluoro (F)-pyrimidines (TriLink Biotechnologies) were also added in a (1:4) ratio and the reaction mixture was adjusted to compensate for the additional volumes. The two RNAs were annealed to form one entity by heating at 94°C for 3
minutes followed by slowly cooling to room temperature within 1 hour. Annealed aptamers were stored in -80°C.
Figure S1. The status of *PRKCI* amplification is not associated with ovarian cancer patient survival or tumorigenic behaviors in established ovarian cancer cells. 

*a*. Overnight-cultured cells were trypsinized and added into 96-well plates and MTT assay was performed at 12 and 84 h after plating. The percent growth was expressed as OD at 84 h relative to value at 12 h. Data are mean ± SD. Mann-Whitney U-test was used to compare the difference between *PRKCI*-amplified and non-amplified groups. NS indicate no statistical significance. 

*b*. Overnight-cultured cells were subjected to Transwell assay. Cells on undersurface of upper chambers were stained and counted using the Imaris 7.0 imaging software. Data are means ± SD. The Mann-Whitney U-test was performed to compare the difference between *PRKCI*-amplified and non-amplified groups. NS indicate no statistical significance.

*c*. Kaplan Meier curve of ovarian cancer patients’ overall survival. 

*d*. Kaplan Meier curve of ovarian cancer patients’ recurrence free survival.
Supplementary Data Figure S2

Figure S2. Silencing PKCι selectively inhibits growth of PRKCI-amplified ovarian cancer cells. PRKCI-amplified cell lines SK-OV-3 and CAOV-3 and non-PRKCI-amplified cell lines OVCAR-8 and IGROV-1 were transfected with 50nM siRNA for 4 days. Images of cells were under a phase-contrast microscope after 4 days of transfection.
Supplementary Data Figure S3

Figure S3. Sensitivity of silencing PKCι to cell growth in OSE and FTECs. Cell growth analysis was performed on OSE and FTECs. The percent growth was expressed as 84-h values relative to 12-h values. The value of PKCι siRNA-treated cells were compared relative to Control siRNA-treated ones, which were normalized to 100%. Data are means ± SD. n = 4. Two-way ANOVA was used to analyze and NS indicates no statistical significance.
**Supplementary Data Figure S4**

**A.** Ovarian cancer cells were infected with lentiviral vector containing Scramble, PKCι or PKCζ shRNA. Cells were detached 4 days of post-infection and then subjected to flow cytometry to analyze cell cycle progression. The horizontal axis is represented by PI (DNA content) and the vertical axis is represented by Count (Intensity). Results are representative of three independent experiments.

**B.** Ovarian cancer cells were infected with lentiviral vector containing Scramble, PKCι or PKCζ shRNA. Cells were detached 4 days of post-infection and subjected to Annexin V/PI-based flow cytometry. The horizontal axis is represented by Annexin V and the vertical axis is represented by PI. Cells in Quadrant 2 and 3 represent apoptotic cells. Results are representative of three independent experiments.

**Figure S4. Knockdown of PKCι induces apoptosis in PRKCI-amplified ovarian cancer cells.** A. Ovarian cancer cells were infected with lentiviral vector containing Scramble, PKCι or PKCζ shRNA. Cells were detached 4 days of post-infection and then subjected to flow cytometry to analyze cell cycle progression. The horizontal axis is represented by PI (DNA content) and the vertical axis is represented by Count (Intensity). Results are representative of three independent experiments. B. Ovarian cancer cells were infected with lentiviral vector containing Scramble, PKCι or PKCζ shRNA. Cells were detached 4 days of post-infection and subjected to Annexin V/PI-based flow cytometry. The horizontal axis is represented by Annexin V and the vertical axis is represented by PI. Cells in Quadrant 2 and 3 represent apoptotic cells. Results are representative of three independent experiments.
Supplementary Data Figure S5

Figure S5. Knockdown of PKC\(\zeta\) induces apoptosis in PRKCI-amplified ovarian cancer cells. Ovarian cancer cells were infected with lentiviral vector containing Scramble, PKC\(\zeta\) or PKC\(\zeta\) shRNA. After 4 days of infection, cells were lused and cell lysates were subjected to Western blotting to detect PKC\(\zeta\), cleaved PARP, cleaved CASP3 and GAPDH with the respective antibodies.
**Supplementary Data Figure S6**

**Figure S6.** Increasing *PRKCI* copies is insufficient to sensitive ovarian cancer cells to PKCι knockdown. 

A. PKCι-containing vector or empty vector was lentivirally introduced into OVCA429 cells. Cells were harvested and cell lysates were subjected to Western blot to detect PKCι. 

B. Empty vector (control) and PKCι-transduced cells (PKCι O/E) were transfected with scramble or PKCι siRNA pool (siPKCι) for 4 days followed by Western blotting to detect CASP3.
Figure S7. Inhibitory effect of Oncrasin, ANF and ATM in ovarian cancer cell lines. Cells were treated with each individual inhibitor at various concentrations. The cell viability was expressed as 84-hour values relative to 12-hour values for each cell line. Data are means ± SD. n = 3. IC50 values were calculated using GraphPad software.
**Supplementary Data Figure S8**

**Figure S8. ANF is cytotoxic to FIECs.** FTECs were treated with Vehicle (DMSO) or ANF at 1µM and images using the microscope at 5X were taken 2 days later.
**Supplementary Data Figure S9**

**Figure S9. Effect of aptamer treatment on intraperitoneal xenograft development.** Once tumor was detected in the mice, they were divided into two groups (5 per group) and received either EpCAM-control or EpCAM-siPKCι aptamer thrice a week intraperitoneally (200nmole/mouse). Tumor outgrowth was monitored weekly using the Xenogen IVIS-200 *In Vivo* bioluminescence imaging system. Error bars represent standard errors. * indicates $P < 0.005$ vs EpCAM control.
Figure S10. Effect of aptamer treatment on body weight of mice. Weights of the mice were measured every week of the experiment and no significant differences were noticed when control and EpCAM-siPKC1 aptamer treated mice were compared. Error bars represent standard errors.
Figure S11. EpCAM aptamer-delivered PKCι siRNA prolongs lifespan of tumor-bearing mice. a. Representative pictures of IHC staining on PKCι, cleaved CASP3 and TUNEL in tumor tissues derived from OCC1 and SK-OV3 cells. Scale bars, 50 µm. b. Kaplan-Meier analysis of animal endpoint survival following treatment with control or EpCAM-siPKCι aptamer in athymic nude mice injected with luciferase-expressing OCC1 cells. n = 5. ** indicates P < 0.01 vs Control Aptamer.
Table S1. Primary and Secondary Antibodies used

| Primary Antibody Name                        | Isotype (IgG) | Company and Catalog Number | MW (kDa) | Dilution Ratio |
|---------------------------------------------|---------------|-----------------------------|----------|----------------|
| Cleaved Caspase 3 (Asp175) (5A1E)           | Rabbit        | Cell Signaling 9664         | 17, 19   | (1:1000)       |
| GAPDH (D4C6R)                               | Mouse         | Cell Signaling 97166        | 37       | (1:2000)       |
| EpCAM/CD326                                 | Mouse         | Cell Signaling 2929         | 40       | (1:1000)       |
| β-Actin (8H10D10)                            | Mouse         | Cell Signaling 3700         | 45       | (1:2000)       |
| Anti-PRKCI                                   | Rabbit        | Millipore Sigma ABC472     | 66       | (1:500)        |
| PKC zeta (C24E6)                             | Rabbit        | Cell Signaling 9368         | 78       | (1:1000)       |
| Cleaved PARP (Asp214) (D64E10)              | Rabbit        | Cell Signaling 5625         | 89       | (1:500)        |

| Secondary Antibody Name                     | Isotype (IgG) | Company | Catalog #   | Dilution Ratio |
|---------------------------------------------|---------------|---------|-------------|----------------|
| IRDye 680RD Secondary Antibody              | Goat anti-Mouse | LICOR   | 926-68070   | 1:20,000       |
| IRDye 800CW Secondary Antibody              | Goat anti-Rabbit | LICOR   | 926-32211   | 1:20,000       |