**Supplementary Materials and Methods and Figure Legends**

**miRNA global profiling real time PCR multiplexing**

Profiling was performed for ARCaPE and ARCaPM PCa cells. miRNA analysis was performed as previously mentioned (1). Each sample was run in duplicates. Three hundred and thirty miRNAs were tested (Ct values, Supplementary Table S1). The raw data was analyzed for significant fold changes. The miRNA with the highest fold changes are depicted on Supplementary Table S2 which were statistically significant (p<0.05, t test).

**Generation of non-integrating human iPSCs using episomal plasmids**

Apparently healthy human fibroblast cell lines (GM05400, 03814 and 02183) were obtained from the Coriell Institute for Medical Research, under their consent and privacy guidelines. All protocols were performed in accordance with the institutional review board’s guidelines at the Cedars-Sinai Medical Center under the auspice IRB-SCRO Protocols, Pro00021505 and Pro00032834. Limbal epithelial stem cell -enriched cultures were prepared from discarded donor corneoscleral rims (01CNL) provided by Drs. Rabinowitz and Maguen within 24 hrs after corneal transplantation, under an approved Cedars-Sinai Medical Center IRB protocol Pro00019393. Cells were isolated by the standard dispase method.Upon iPSC generation at Cedars Sinai, they were renamed 00iCTR-n2, 14iCTR-n6, 83iCTR-n1, and01iCNL-n1 to reflect catalog or identification numbers, control line and clone number (2, 3). Fibroblasts or limbal cells were reprogrammed into virus-free iPSC lines using the Amaxa Human Dermal Fibroblast Nucleofector Kit to express episomal plasmids with 6 factors: *OCT4, SOX2*, *KLF4*, *L-MYC*, *LIN28*, and *p53* shRNA (Addgene) (4). This method has a significant advantage over viral transduction, because exogenously introduced genes do not integrate and are instead expressed episomally in a transient fashion. Briefly, fibroblasts (0.8 x 106 cells per nucleofection) were harvested, centrifuged at 200*g* for 5 minutes, re-suspended carefully in Nucleofector® Solution (VPD-1001, Lonza) and the U-023 program was applied. All cultures were maintained under norm-oxygen conditions (5%O2) during reprogramming, which further enhance the efficiency of iPSC generation. The media was kept on for 48 hours and gradually changed to chemically-defined mTeSR®1 medium containing small molecules to enhance reprogramming efficiency. The small molecules used were, (1) sodium butyrate (0.5 mM; Sigma-Aldrich), (2) glycogen synthase kinase 3β inhibitor of the Wnt/β-catenin signaling pathway (CHIR99021, 3 µM; Tocris Bioscience/ R&D Systems, Minneapolis, MN), (3) MEK pathway inhibitor (PD 0325901, 0.5 µM; (Stemgent, Cambridge, MA), (4) Selective inhibitor of TGF-β type I receptor ALK5 kinase, type I activin/nodal receptor ALK4 and type I nodal receptor ALK7 (A 83-01, 0.5 µM; (Tocris Bioscience). Colonies with ES/iPSC-like morphology appeared 25-31 days later. Subsequently, colonies with the best morphology were transferred onto a feeder-independent BD Matrigel™ Matrix and maintained in mTeSR®1 medium (5).

**Human embryonic stem cell (ESC) and iPSC cell culture**

Human ESC line, H9 (WiCell, Madison, WI) and iPSC lines were maintained onto a feeder-independent BD Matrigel™ Matrix and maintained in mTeSR®1 medium. Colonies grown on growth factor-reduced Matrigel (BD Biosciences, San Jose, CA) had typical ESC-like morphology with well-defined borders, and high nuclear/cytoplasmic ratio. The iPSC clones were further expanded and cryopreserved according to previously published protocols (5, 6).

**Human iPSC characterization**

Human iPSCs were rigorously characterized at the Cedars-Sinai iPSC core using several assays. G-Band karyotyping (see below) ensured normal a karyotype, and genomic DNA PCR confirmed the absence of episomal plasmid genes, as previously described (3, 4, 7). Pluripotency was assessed by immunostaining with surface and nuclear pluripotency markers for subsequent flow cytometry quantification (> 80% SSEA4 and Oct3/4 double positivity), by quantitative RT-PCR of endogenous pluripotency genes, and by gene-chip and bioinformatics-based PluriTest assays. Spontaneous embryoid body differentiation confirmed the capacity to form all germ layers. Characterization of iPSC lines used in this study has been previously published (3, 8).

**3`UTR assay**

 293T cells were stably transduced with Lenti Goclone lentivirus particles containing a constitutive promoter driving a hybrid luciferase-3` UTR of human STAG2 transcript (MISSION® 3′UTR Lenti GoClone™, Sigma-Aldrich). Cells were selected using puromycin. Mimics of miR-409-5p, miR-409-3p and control miRNA were transiently transfected into these 293T cells and luciferase activity was determined using Lightswitch luciferase assay system (Switchgear genomics).

RSU1 mutant luciferase activity: 3`UTR construct (Switchgear genomics) was used as the wild type (WT) luciferase construct and it was further mutated as described below. miR-409-5p mimic and control miRNA were transiently transfected along with the WT or mutant (RSU1) construct into these 293T cells and luciferase activity was determined 24 h later using Lightswitch luciferase assay system (Switchgear genomics).

**3`UTR Mutant constructs:** Mutated 3’ UTR luciferase constructs were produced by sited-directed mutagenesis. Briefly, primer pairs with two sequential base pair mutations in the miRNA seed sequence of the 3’ UTR were generated. Following polymerase chain reaction amplification, parental methylated template DNA was digested for 1 hour with *Dpn* I. 2 µl of the reaction was then transformed into XL-10 Gold bacteria. 16 hours post-transformation, colonies were picked for liquid culture. Plasmid DNA was isolated by the Zyppy Plasmid Miniprep Kit according to manufacturer’s directions (Zymo Research). Mutations were confirmed by sequencing before proceeding with luciferase assays.

**Primers**

|  |  |
| --- | --- |
| miR-409-5p RSU 1 | gttaacagtgacatttaaatggggacatgattttaattattcttttgataataagcaaccttg |
| miR-409-5p RSU 2 | caaggttgcttattatcaaaagaataattaaaatcatgtccccatttaaatgtcactgttaac |

**Antibodies used for IHC and Western Analysis**

Antigen retrieval was used for IHC.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Secondary | Dilution | Company |  Catalog |  Reactivity |
| NANOG | mouse | 1:50 | Santa Cruz Biotechnology | sc-134218 | h,m |
| OCT3/4 | mouse | 1:50 | Santa Cruz Biotechnology | sc-5279 | h,m |
| CK8 | mouse | 1::200 | Covance, Inc | MMS-162P | h,m |
| CK5 | rabbit | 1::200 | Covance, Inc | PRB-106p | h,m |
| p-Akt | rabbit | 1:50 | Cell Signaling | 4060S | h,m |
| Ki-67 |  rabbit |  1:100 | Abcam | ab16667 |  h,m |
| STAG2 | rabbit | 1::200 | Cell Signaling technologies | 5882 | h,m |
| RSU1 | rabbit | 1::200 | Proteintech group | 11207-1-AP | h,m |
| VIM | mouse |  1:200 | Santa Cruz Biotechnology | sc-6260 |  h,m |
|  |  |  |  |  |  |
| E-cadherin | rabbit | 1:500 | Cell signaling | 3195 | h.m |

**miRNA determination from exosomes**

Cancer cells were maintained in T-medium with exosome depleted FBS media supplement (System Biosciences) for 48 h and conditioned media was used to extract exosomes. Exo-Quick –TC (System Biosciences) for tissue culture Media, and SeraMir exosome RNA purification kit (System Biosciences) was used to extract miRNA from exosomes. MiRNA were detected for miR-409-5p/-3p by qRT-PCR analysis.

**Supplementary Figures**

**Supplementary Table. S1**

Gleason scores of the cancer from which the TMA cores were obtained and the miR-409 intensity counts by ISH-QD. None of the TMA cores had any treatment.

**Supplementary Table. S2**

Global miRNA expression, represented as Ct values assayed by multiplexed qRT-PCR analysis in ARCaPE and ARCaPM PCa EMT bone metastatic model.

**Supplementary Table. S3**

miRNA differentially expressed in metastatic ARCaPM prostate cancer cells and non-metastatic ARCaPE prostate cancer cells by multiplexed real time PCR analysis. miRNA in bold are in the DLK1-DIO3 mega-cluster.

**Supplementary Fig. S1**

A, miR-409-5p and miR-409-3p binding sties in 3`UTR of RSU1 mRNA. B, Effect miR-409-5p mimic binding on 3` UTR of RSU1 luciferase construct, both wild type and mutated construct. Effect of miR-409-5p mimic and miR-409-3p mimic on STAG2 3`UTR luciferase construct measured by luciferase assay.

**Supplementary Fig. S2**

Effect of miR-409 overexpression in LNCaP cells. A, miR-409-5p and miR-409-3p levels in LNCaP control cells (LNCaP-C) and LNCaP miR-409 expressing cells (LNCaP-409) measured by qRT-PCR. B, Protein expression of RSU1 in LNCaP-C and LNCaP-409 PCa cells. C, Protein expression of E-cadherin in ARCaP-409 and LNCaP-409 PCa cells compared to their controls, measured by western analysis.

**Supplementary Fig. S3**

A, Nanog and Oct-3/4 expression in tumor and stromal areas of prostates expressing miR-409 compared to control normal prostates, using IHC analysis. B, IHC staining of cytokeratin 8 (CK-8) and cytokeratin 5 (CK-5) in normal control prostate, and tumors of control and miR-409 expressing prostates.

**Supplementary Fig. S4**

A, miR-409-5p and miR-409-3p in exosomes from ARCaPE and ARCaPM PCa cells measured by qRT-PCR. B, miR-409-5p levels in exosomes from ARCaPM-C and ARCaPM-409-5pi PCa cells measured by qRT-PCR.

**References:**

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