Reprogramming of Prostate Cancer-Associated Stromal Cells to Embryonic Stem-Like

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BACKGROUND. $CD90^+$ prostate cancer-associated (CP) stromal cells represent a diseased cell type found only in tumor tissue. They differ from their normal counterpart in gene expression and inductive signaling. Genetic reprogramming by induced pluripotent stem (iPS) cell technology can effectively change adult cells into stem-like cells through wholesale alteration of the gene expression program. This technology might be used to 'erase' the abnormal gene expression of diseased cells. The resultant iPS cells would no longer express the disease phenotype, and behave like stem cells.
METHODS. CP stromal cells, isolated from tumor tissue of a surgically resected prostate by anti-CD90-mediated sorting and cultured in vitro, were transfected with in vitro packaged lentiviral expression vectors containing stem cell transcription factor genes POU5F1, LIN28, NANOG, and SOX2.
RESULTS. Alkaline phosphatase-positive iPS cells were obtained in about 3 weeks post-transfection at a frequency of 10^{-4}. Their colony morphology was indistinguishable from that of human embryonic stem (ES) cells. Transcriptome analysis showed a virtually complete match in gene expression between the iPS and ES cells.
CONCLUSIONS. Genes of CP stromal cells could be fully inactivated by genetic reprogramming. As a consequence, the disease phenotype was 'cured'. *Prostate* © 2012 Wiley Periodicals, Inc.

KEY WORDS: **prostate cancer-associated stromal cells; induced pluripotent stem cells; transcriptomes**

INTRODUCTION

Induced pluripotent stem (iPS) cells can be obtained from various somatic cell types by the introduction of particular stem cell genes encoding potent transcription factors (TFs) [1,2]. This technology can apparently replace the gene expression of a cell with that of stem cells. Can cell types of diseased tissue be reprogrammed? In this way, (mutated) genes responsible for the disease phenotype may be silenced, thus providing a possible means of cure, for example, in

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the case of terminal prostate cancer. Additionally, we do not generally know all the genes directly responsible for many diseases. For that reason, therapeutic targeting of single identified genes has limited efficacy. The iPS strategy, however, targets the entire gene repertoire and is able to silence many genes simultaneously. Since phenotype is determined by the genotype, this genotype rewriting could, as a result, eradicate the diseased phenotype. The derived stem-like cells, furthermore, could respond to normal differentiative signaling. Recently, Hu et al. [3] showed that chronic myeloid leukemia (CML) cells could be converted to iPS cells. The resultant cells retained the CML chromosomal rearrangement but were capable of differentiating into multiple hematopoietic cell types. Similarly, could iPS cells be generated from the diseased cell types in a solid tissue like the prostate? In this report, we used iPS technology to reprogram the prostate cancer-associated (CP) stromal cells found in primary tumors to test this idea. These tumor-restricted cells, though not regarded as malignant as the cancer epithelial cells, differ from their normal counterpart in the expression of multiple genes [4] and are not as effective in stromal induction of stem cell differentiation [5]. These cells are not derived from mesenchymal stem cells based on flow cytometry and gene expression analyses [6]. Unlike the cancer epithelial cells, stromal cells can be readily grown in culture [7,8] to produce sufficient numbers for reprogramming given the low efficiency of current gene transfection protocols.

The major cell types in a surgically resected human prostate are luminal epithelial, basal epithelial, stromal fibromuscular as well as the tumor counterpart of cancer epithelial and cancer-associated stromal cells. Leukocytic, endothelial, and nerve cells are other types in fair abundance [9,10]. These major cell types can be isolated after collagenase digestion of tissue into single cells, followed by density gradient centrifugation and magnetic cell sorting (MACS) using antibodies to cell-type specific cluster designation (CD) cell surface antigens [4,11–13]. The cancer stroma is well marked by strong immunostaining for CD90/THY1 [4,14]. Thus, the $CD90^+$ CP stromal cells were sorted from fresh tumor-enriched tissue specimens obtained from surgery, and grown in vitro. Identity of the cultured cells was checked by gene expression analysis. The cultured CP stromal cells were reprogrammed by transfection with lentiviral vectors containing gene constructs of human stem cell TFs POU5F1/OCT4, NANOG, SOX2, LIN28.

To characterize the CP stromal-derived iPS cells, their transcriptome was determined by Affymetrix DNA microarray analysis. The transcriptome dataset was then compared with those of stem cells as represented by the human embryonic stem (ES) cell line H1 (WA01) [15] and the embryonal carcinoma (EC) cell line NCCIT [16].

MATERIALS AND METHODS

Isolation of Human CP Stromal Cells

Isolation of stromal cells from prostate tissue was carried out as described [4]. Excess tissue samples from surgically resected glands were approved for research purposes by the University of Washington IRB with written informed patient consent. Typically, 0.3–0.7 g of tissue samples were processed to obtain enough sorted $CD90^+$ cells for transcriptome analysis and in vitro culture. Corresponding frozen sections were histologically examined to confirm the phenotype of the selected specimens. The tumor sample used in this study was obtained from case 09-124CP—a transition zone tumor of Gleason score $4 + 3$, staged T2c. For non-cancer (NP), samples were obtained from the non-involved part of resected prostates. Tissue samples were minced and digested by overnight incubation with 0.2% collagenase type I (Invitrogen, Carlsbad, CA) in RPMI1640, 10^{-8} M dihydrotestosterone on magnetic stirrer at room temperature. The resultant cell suspensions were filtered, diluted with equal volume of Hanks balanced salt solution, and aspirated. Cells were pelleted by centrifugation and partitioned into stromal and epithelial fractions on discontinuous Percoll density gradients (Amersham Pharmacia, Piscataway, NJ). Stromal cells were collected, sorted by MACS with R-phycoerythrin (PE)-conjugated anti-CD90 for CP and anti-CD49a for NP followed by anti-PE conjugated magnetic beads.

The sorted cells were passaged in RPMI1640, 10% fetal bovine serum (FBS), gentamycin [17] and their identity was checked by reverse transcriptase polymerase chain reaction (RT-PCR) analysis as well as Affymetrix GeneChip arrays as described [7,18]. Their biology was assessed by co-culture with NCCIT for stromal induction of stem cell differentiation [13,19]. Cultured stromal cells were harvested by trypsin digestion and plated for gene transfection.

Lentiviral Transfection of Stromal Cells

The cultured stromal cells were infected with two lentivirus vectors, one encoding viral peptide 2A-linked POU5F1 and SOX2, and the other encoding 2A-linked LIN28 and NANOG. The peptide 2A sequences allowed intrinsic cleavage of the linked human proteins [20]. The expression plasmids, pSIN-EF2-O2S and pSIN-EF2-N2L [21], were purchased from Addgene (Cambridge, MA). Production and

concentration of lentiviral vectors were supplied by the Core Center of Excellence in Hematology (CCEH), Viral Vector Production Core at the Fred Hutchinson Cancer Research Center (Seattle, WA). Transient transfections of 293T producer cells were performed with second generation lentiviral packaging [22,23], whereby transfection mixes were comprised of modified helper plasmids expressing VSV-G and the lentiviral Gag, Pol, Rev, and Tat genes. Approximately 1.4×10^7 293T cells were plated on 150-mm culture dishes, and each preparation was transfected with 6 mg VSV-G plasmid, 17.5 mg Gag-Pol plasmid, and 27 mg each of the pSIN plasmids in the presence of 2 ml serum-free DMEM and 151 mg polyethyleneimine. Viral harvest media was supplemented with 10 mM sodium butyrate and 1 mM HEPES, pH 7.0 for the first harvest, and with 1 mM HEPES, pH 7.0 for subsequent harvests. The viral vectors were concentrated 100-fold by centrifugation at 7,200*g* prior to resuspension in Iscove's modified Dulbecco media.

Lentiviral vector transduction was performed in 4 μg/ml polybrene under hypoxia (5% O_2, 5% CO_2) in DMEM supplemented with 20% FBS with 25 μl virus stock added to cells plated on 35-mm dishes for 1 day. Media was changed on day 2, and the cells were plated onto 100-mm dishes coated with gelatin. On day 6, the cells were plated at different densities (ranging from 10^4 to 10^5) on 100-mm dishes in human ESC media F12/DMEM with KO Serum Replacer (Invitrogen, Carlsbad, CA) containing the histone deacetylase (HDAC) inhibitors Na butyrate and SAHA (suberoylanilide hydroamic acid), and irradiated mouse embryonic fibroblasts (MEF). MEF cells were prepared from pregnant female mice at E12.5. The embryos were guillotined, liver and heart removed, and dispersed by repeated passages through a syringe needle. Cells were cultured and passaged in MEF media (high glucose DMEM with FBS) to 4×10^6 per 100 mm plates, harvested and irradiated. iPS colonies were identified by the unique morphology of stem cell growth. Candidate iPS colonies were verified by staining for alkaline phosphatase (ALP) [19]. Individual clones were selected for expansion and stored frozen in liquid N_2. Cell colonies were imaged by Zeiss observer A1 camera (AxioCam MRC, Thornwood, NY) using Axiovision software. The efficiency of stromal cell reprogramming was compared with that of H1SF cells (SF, self feeder), which were fibroblast-like that spontaneously arose from cultures of H1 cells. Curiously, not all 'self-feeders' were as readily reprogrammed.

Viral Infection Analysis

For viral infection and transgene expression, transfected cells were analyzed by RT-PCR. Coding region sequence (CDS) primers (Table I) were used to detect transcripts of NANOG, SOX2, LIN28, and POU5F1 in infected cells. 3′UTR (untranslated region) primers were used to detect expression from the corresponding endogenous genes, i.e., as a result of the activation of the stem cell program by the introduced gene constructs. These primers were designed not to recognize expression from the vectors as the primer sequences were missing in the transgenes. Primers for PROM1 (CD133) and β2-microglobulin (B2M) were also used, the latter as reaction control. About 500 ng RNA was converted to cDNA for PCR amplification. The primer

TABLE I. PCR Primers

3′UTR primers			
5-NANOG	CAGTCTGGACACTGGCTGAA	1164–1183	NM_024865.2 CDS: 217–1134
3-NANOG	CTCGCTGATTAGGCTCCAAC	1293–1312	
5-SOX2	GCTAGTCTCCAAGCGACGAA	1804–1823	NM_003106.2 CDS: 428–1381
3-SOX2	GCAAGAAGCCTCTCCTTGAA	1928–1947	
5-POU5F1	GACAGGGGGAGGGGAGGAGCTAGG	1495–1518	NM_203289.4 CDS: 897–1469
3-POU5F1	CTTCCCTCCAACCAGTTGCCCCAAAC	1613–1638	
CDS primers			
5-NANOG	CTCACCTATGCCTGTGATTTGTGGGC	281–307	
3-NANOG	GCTGGAACTGCATGCAGGACTGCAG	958–982	
5-LIN28	CCGTGTCCAACCAGCAGTTTGCAG	122–145	NM_024674.4 CDS: 115–744
3-LIN28	GAGCAGGGTAGGGCTGTGGATTTC	703–726	
5-PR0M1	CATCCACAGATGCTCCTAAGGCTTGG	283–308	NM_006017.2
3-PR0M1	GCTGTGTACTTTGTTGGTGCAAGCTC	1046–1071	
5-B2M	GGCTATCCAGCGTACTCCAAAGATTC	117–142	NM_004048.2
3-B2M	GTCTCGATCCCACTTAACTATCTTGGGC	387–414	

The genes and gene primer pairs (5- and 3-) are tabulated in the first and second column, respectively. In the third column are the sequence co-ordinates to the gene entries identified by accession number in the fourth column. The 3′UTR primers are derived from sequences outside the coding region (CDS).

annealing temperature was 62°C except, 64°C for NANOG and 60°C for B2M. PCR was carried out for 25 cycles, and the products were analyzed by agarose gel electrophoresis and stained by ethidium bromide.

ALP Staining

Cultured cells were fixed in 70% ethanol for 1–2 min. The Vector Black ALP substrate solution was prepared according to the manufacture's protocol (Vector, Burlingame, CA) in 100 mM Tris-HCl, pH 9.5. The reaction product was brown/black.

RNA Isolation

iPS cells from clones were stored in freezing media (12.5% DMSO in hESC media) at 1 ml per 100-mm plate for five straws. Each straw was loaded with a small amount of freezing media, a small air bubble, and 200 μl cells. For isolation of RNA, iPS cells on culture dishes were treated with dispase, pelleted and replated on Matrigel in TeSR2 media (Stemcell Technologies, Vancouver, Canada), which helped to maintain iPS cell in the undifferentiated state. Total RNA was isolated from the harvested iPS cells using mirVana (Applied Biosystems/Ambion, Austin, TX). RNA concentration was determined by ND1000 spectrophotometer (NanoDrop, Wilmington, DE), and RNA quality was checked by Agilent 2100 Bioanalyzer RNA Nano Labchips (Agilent Technologies, Santa Clara, CA). The RNA was analyzed by Affymetrix Human Genome U133 Plus 2.0 GeneChips (Affymetrix, Santa Clara, CA), which contained probesets representing 54,675 mRNA genes, splice variants, and expressed sequence tags (ESTs).

DNA Microarray Hybridization

For mRNA expression of iPS cells, 200 ng RNA was reverse transcribed with poly (dT) primer/T7 promoter, and the cDNA was made double-stranded. In vitro cDNA transcription was performed to produce unlabeled cRNA. Next, first-strand cDNA was again produced with random primer/T7 promoter followed by in vitro transcription to produce biotinylated cRNA probes. The GeneChips were prepared, hybridized, and scanned according to the protocols provided by Affymetrix [12]. Array datasets for stem cells were also previously obtained from H1 and NCCIT [24]. All microarray data were MIAME compliant. The iPS dataset was deposited in GEO, accession number GSE35373.

Microarray Data Analysis

The array results were normalized with global mean [12,25]. Array data analysis was described previously [4,19]. Gene expression levels were defined as the normalized and summarized intensities of each GeneChip probeset, and presented as the logarithmic value: $X = \log_2(\text{Normalized intensity})$. This step was carried out by the standard robust multi-array average method [25], implemented in the in-house analysis pipeline SBEAMS [26] at the Institute for Systems Biology (Seattle, WA). An in-house designed analysis software was used to group genes into clusters based on fold-change and normalized intensity, and then to cross-compare these datasets. Principal components analysis (PCA) of transcriptome datasets was described in full by Pascal et al. [19]. The R-language script and an illustrative dataset example for PCA were provided at http://labpib.openwetware.org/PCA.html. A PCA subspace was first created with the transcriptomes of four cell types isolated by MACS from the human prostate: $CD26^+$ luminal, $CD104^+$ basal, $CD49a^+$ stromal, and $CD31^+$ endothelial [19]. Transcriptome datasets of other cell types were then projected into this prostate PCA plot [24]. Datasets whose placements were near each other would signify a close similarity in overall gene expression by the corresponding cell types. Thus, ES and EC cells were well apart from the four differentiated cell types but proximal to each other to indicate their similar gene expression as reported by Sperger et al. [27]. PCA also showed the expression difference between $CD90^+$ CP stromal and $CD49a^+$ NP stromal cells [4]. The dataset of the generated iPS cells from CP stromal was projected into the PCA space to visualize the extent of reprogramming.

RESULTS

Viral Transfection and Reprogramming Efficiency of CP Stromal Cells

For reprogramming, cultured CP stromal and NP stromal were transfected with NANOG, SOX2, LIN28, POU5F1 expression vectors. The transfected stromal cells were harvested and analyzed by RT-PCR. Figure 1A shows that both NP stromal and CP stromal were successfully infected and the transgenes were expressed. Uninfected cells were negative. The data shown for NANOG and LIN28 indicated an equal level of expression of these two genes based on stain intensity of the product bands. This resulted from these two transgenes being linked on the same expression vector. At the time of analysis, the cells were not yet reprogrammed because the endogenous counterparts of these TF genes were not activated as in the reprogrammed H1SF cells used as control (Fig. 1B). There was no detectable expression for NANOG, SOX2, POU5F1, plus PROM1/CD133 in these infected stromal cells using primers designed to

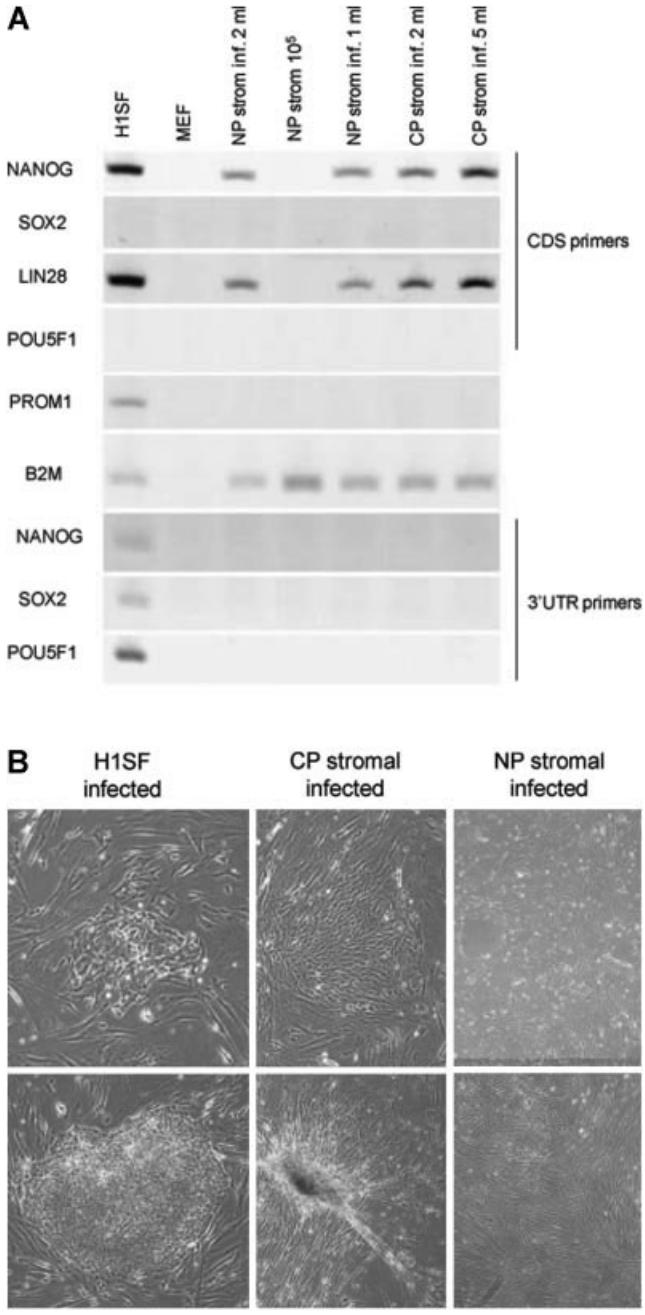


Fig. I. Lentiviral transfection of human prostate cells. **A**: RT-PCR analysis showed both NP stromal and CP stromal were infected as indicated by the CDS primers but not yet reprogrammed as indicated by the 3′UTR primers. The positive control was HISF. Human B2M is absent in MEF cells. Reactions involving the SOX2 and POU5FI CDS primers failed; it could not be ruled out that these cells were not infected by the POU5FI-SOX2 vector. **B**: Shown are the morphologies of the HISF, CP stromal, NP stromal cultures before (top row) and after (bottom row) viral infection at the time of RT-PCR analysis.

detect transcripts off the chromosomal gene loci. It was also possible that viral infection was suboptimal since POU5E1 and SOX2 transgene expression was not detected, and that the H1SF cells required fewer TF genes for reprogramming than the prostate stromal cells.

To enhance the transfection efficiency, CP stromal was infected with a higher viral titer under hypoxia with HDAC inhibitors and MEF as feeder. At day 13 post-infection, incipient iPS colonies were observed (Fig. 2A). At day 18, colonies (clones) were picked and expanded. iPS colonies were obtained from four platings of CP stromal with the following frequencies: $18/10^5$ (0.02%), $4/5 \times 10^4$ (0.01%), $1/2 \times 10^4$ (0.005%), and $2/10^4$ (0.02%) for a total of 25 colonies. The iPS cells were stained positive for the stem cell marker ALP (Fig. 2A); CP stromal was negative for ALP. The colony morphology of iPS09-124CPstrom (clone #5) appeared indistinguishable from that of human ES cells in culture (Fig. 2B).

Gene Expression Analysis of CP Stromal-Derived iPS Cells

iPS clone #5 was expanded in TeSR2 media and harvested for gene expression analysis. The Affymetrix U133 array was used because all cell type-specific transcriptomes were previously obtained with this GeneChip array [24]. The iPS cell transcriptome was analyzed and plotted in the PCA subspace defined by the transcriptomes of luminal, basal, stromal, and endothelial cells. Figure 3A shows the placement of the iPS09-124CPstrom dataset, (X20101208_01_ips_CL.CEL) as deposited in The Urologic Epithelial Stem Cell Database (UESC, http://scgap.systemsbiology.net/), in relationship to the differentiated cell types. Notably, it was about equally distant from the stromal as the other three cell types. In Figure 3B, the datasets of two $CD90^+$ CP stromal cell populations (CP stromal 1 and 2) sorted from two different specimens 08-028CP and 08-032CP [4], and cultured CP stromal specimen 08-021CP were plotted to show the large datapoint separation between these stromal cell types and iPS09-124CPstrom. Cell culturing is known to induce gene expression changes [17], and this was reflected in the separation between sorted and cultured stromal cells in addition to their being isolated from tumors with different Gleason scores [4,19]. The iPS dataset was also compared with that of ES (H1) and EC (NCCIT) as shown in Figure 3C. By the distance unit between datapoints, the iPS cells were more similar to ES than EC (which was established from a germ-cell tumor). By PCA (data not shown), the iPS was also less similar to a putative progenitor

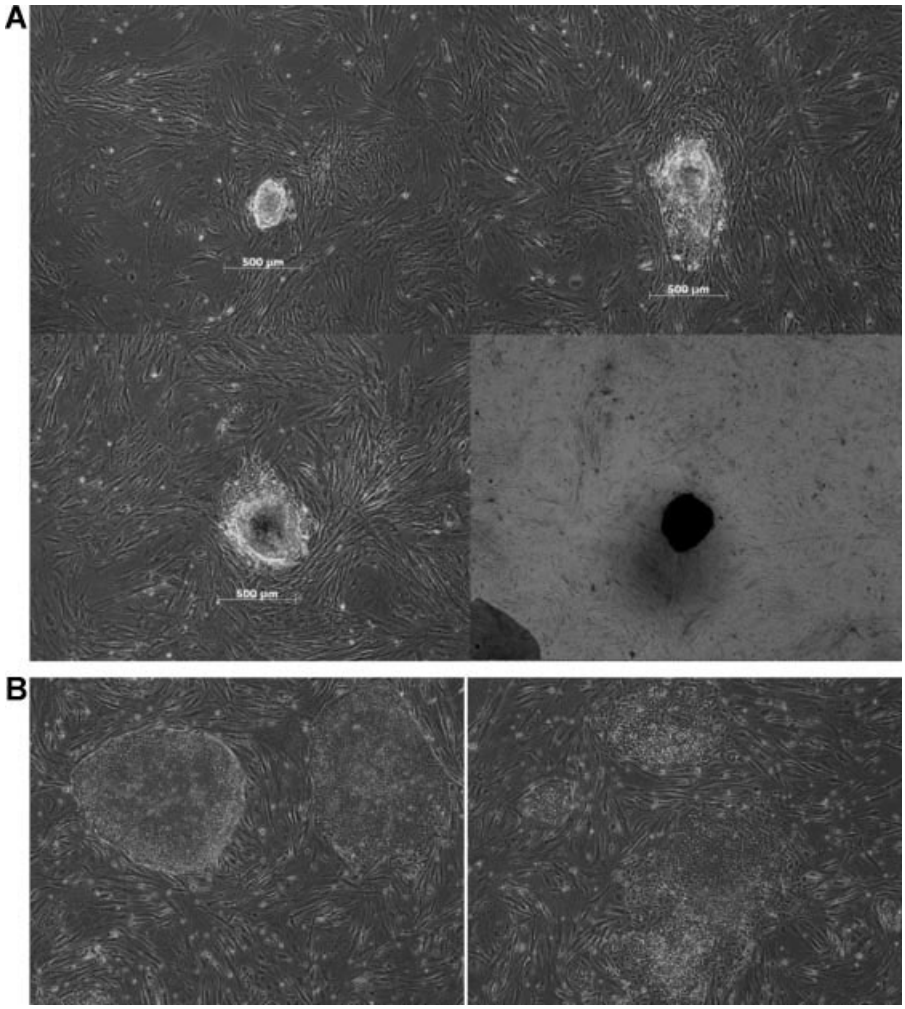
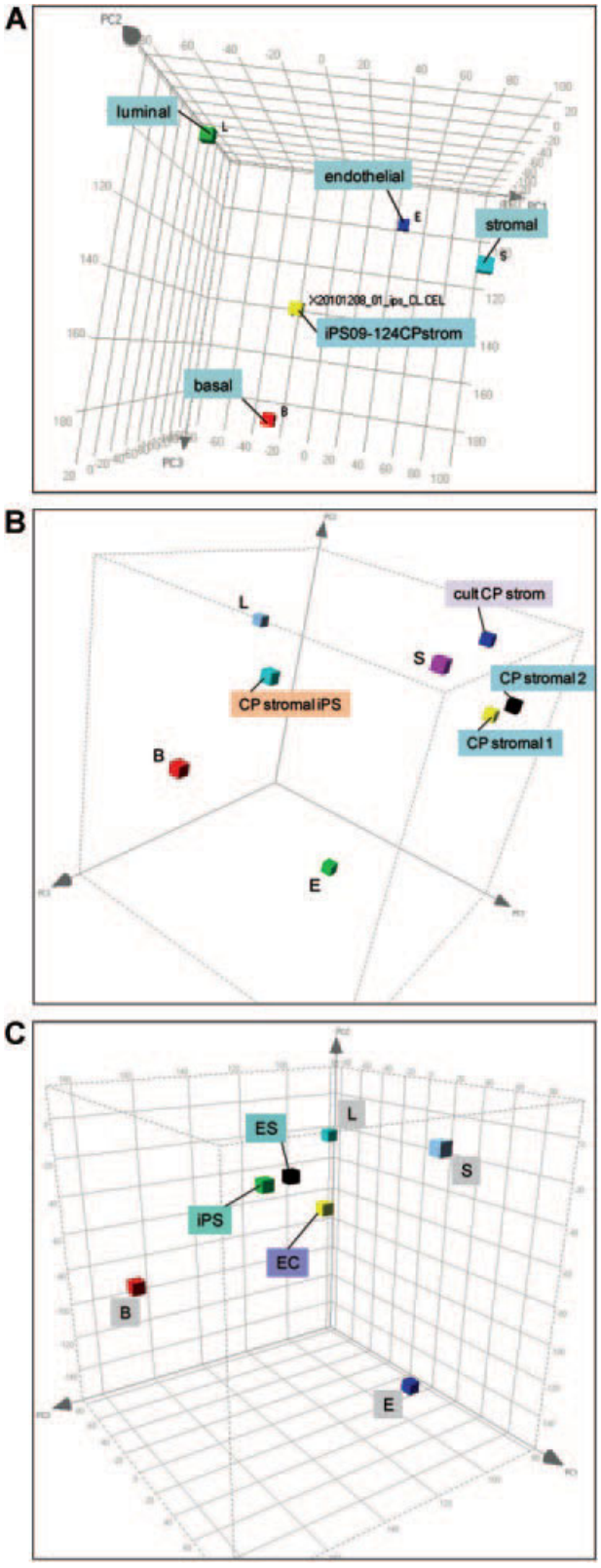


Fig. 2. iPS colonies derived from 09-124CP stromal. **A**: Three individual colonies and an ALP-positive colony (lower right panel) are shown. **B**: Two iPS colonies (left photomicrograph) exhibit similar morphology to that of ES cells. One colony in the right photomicrograph shows cells with 'differentiated' morphology on the periphery. Cells in the background are MEF.

cell type isolated from the prostate via expression of the membrane transporter ABCG2 [24,28].

Gene Expression Similarity Between iPS and ES cells, and Inactivation of CP Stromal Gene Expression in Reprogramming

For comparison of the expression levels of informative genes, transcriptome datasets archived in UESC were interrogated. Use of the database for query as well as assessment of the array data quality have been described [29,30]. Figure 4A panel A shows query results for the newly derived iPS cells. Expression of POU5F1, NANOG, LIN28, SOX2, CD133 was at equivalent levels based on array signal intensity values on a gray scale in both the iPS and ES (and EC) cells. The differential levels of the TF genes were more or less the same between ES and iPS, an indication of endogenous expression and not of continuous expression from the viral transgenes. The RT-PCR result of reprogrammed H1SF cells in Figure 1A was concordant with the array data showing expression level of POU5F1 higher than those of NANOG, SOX2, and PROM1 (3′UTR primers) based on gel band intensities. These genes were not found in the datasets for sorted CP stromal 1 and 2, as well as cultured CP stromal. CD90 expression is elevated in the CP stroma, and its expression level was unchanged in the iPS as it is also a stem cell marker. Figure 4A panel B shows that expression of the CP stromal genes ACTA2 (smooth muscle actin), MMP9 (matrix metalloproteinase 9), STC1 (stanniocalcin 1), TNC (tenascin) was down-regulated to the levels in ES cells. MMP9 is differentially up-regulated in CP stromal cells versus NP



stromal cells [4,5]. The other genes are characteristic of prostate stromal cells [18], which are phenotypically smooth muscle cells. These results verified the PCA of transcriptome datasets.

Figure 4B panel A shows an MA-plot of the iPS and ES transcriptomes. Of the total number of genes represented on the Affymetrix GeneChips, only some 20 showed more than twofold expression differences between iPS and ES. Many of these were unknown ESTs. The query result for MAGEA2, LOC339260, CXCL11, ZNF560 is shown in Figure 4B panel B, the first three were higher in expression in ES. ZNF560 detected in the iPS cells was not expressed in the stromal cells (CP or NP). Therefore, essentially all CP stromal genes were silenced as a result of reprogramming to be replaced by genes specific to stem cells. The transcriptome data analysis provided clear evidence that full reprogramming of CP stromal was achieved by lentiviral transfection, and that the resultant cells were ES-like.

DISCUSSION

$CD90^+$ CP stromal cells are abnormal, and are restricted to primary tumors in which they surround the cancer glands [14]. Here, we showed that the CP stromal cells of tumor case 09-124 can be fully reprogrammed by the forced expression of four stem cell TF genes. The derived iPS cells displayed the colony morphology of ES cells. Reprogramming was furthermore assessed by PCA of their transcriptome, which showed that the gene expression matched that of ES cells, and less that of EC cells (which could be regarded as the cancer counterpart of ES cells). The expression levels of many genes were equivalent in the iPS and ES cells, as in the case of POU5F1, LIN28, SOX2, and NANOG, which are essential for maintenance of the stem cell phenotype. At the same time, genes of CP stromal cells were down-regulated. Since this was the objective of our study, we did not perform mouse xenograft of these cells for teratoma formation to assay pluripotency [31]. Thus, iPS

Fig. 3. Transcriptome of 09-124CP stromal-derived iPS cells. **A**: This PCA subspace is defined by the transcriptomes of luminal L, basal B, stromal S, endothelial E cells isolated from the prostate. The iPS transcriptome was projected into this space. The placement of the datapoint, X20101208.01.ips.CL.CEL, shows that the iPS cells are unlike any of the four differentiated cell types. PCI, PC2, and PC3 are the three principal components axes. **B**: This PCA display shows the relative positioning of the NP stromal (labeled S), CP stromal 1 and 2 and cult(ured) CP stromal datapoints with respect to the iPS datapoint. **C**: This PCA display shows the relatedness between iPS and ES versus EC. The shorter distance unit of separation between iPS and ES than that between iPS and EC indicates a closer overall gene expression.

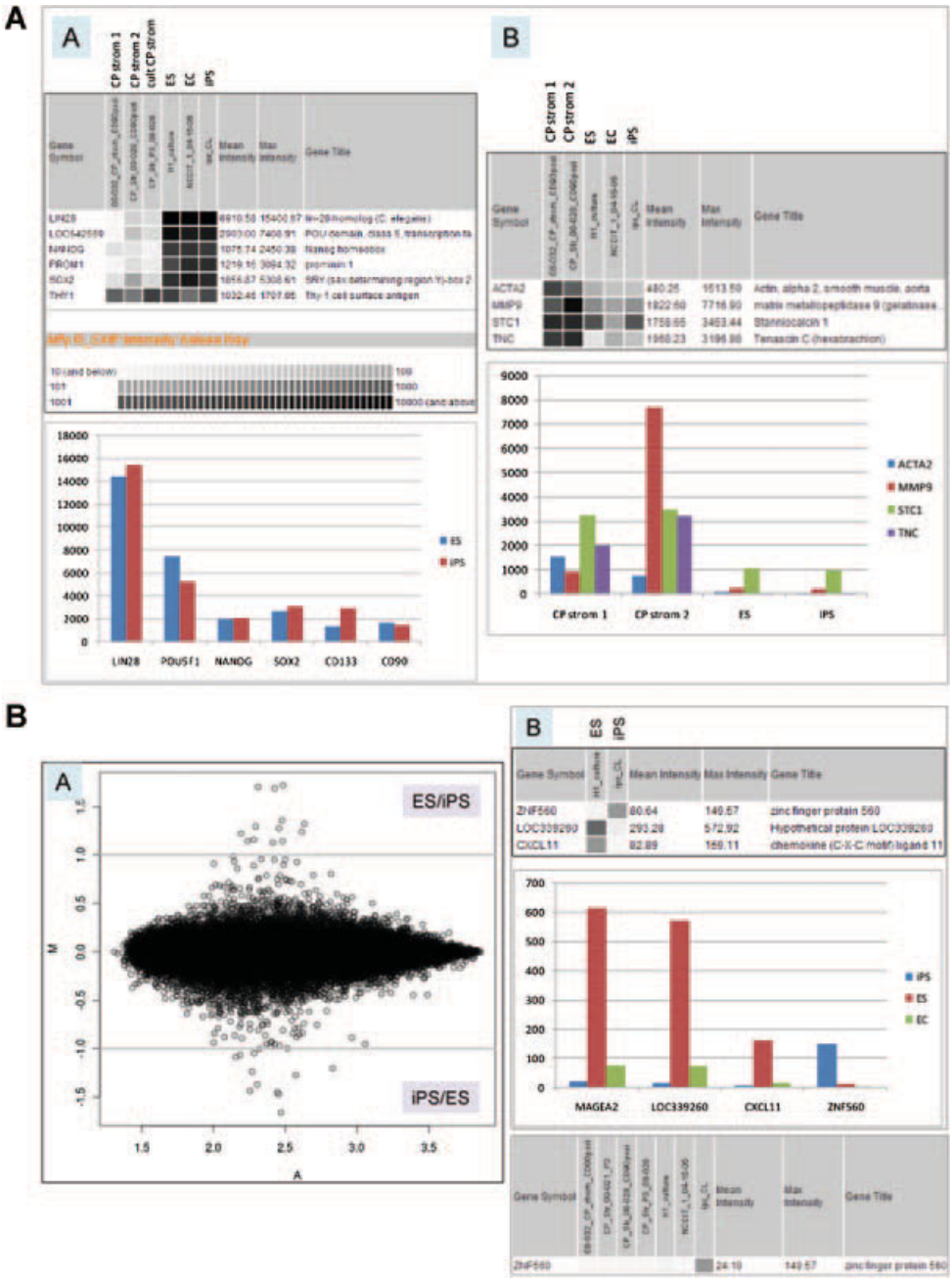


Fig. 4. Dataset query. **A**: Panel A shows the expression levels of LIN28, POU5FI (LOC642559), NANOG, PROMI, SOX2, THYI in sorted CP stromal and cultured CP stromal versus that of iPS (and ES, EC) as detected by DNA array analysis on a gray scale. The array signal levels are also plotted in a histogram format. Panel B shows the expression levels of selected CP stromal genes. Note the near identical pattern for these genes in iPS and ES. The CP stromal cells were sorted from different Gleason score tumors, hence the variable levels of these genes in the two populations. **B**: Panel A shows the MA-plot to indicate that gene expression is essentially alike with only about 20 genes differentially expressed by >2-fold. Panel B shows the dataset query display for several examples. Note for three, the levels are closer between iPS and EC. ZNF560 in the iPS cells is not expressed by CP stromal cells.

technology can silence multiple gene targets in a particular diseased cell type. Work is undergoing to see if CP stromal cells isolated from multiple tumor samples (with different Gleason scores) and other prostate cell types can be reprogrammed at the same efficiency. As can be seen in Figure 4A (panel B), CP stromal cells do not have uniform gene expression. These results would determine variability in gene expression, if any, of iPS cells derived from multiple sources.

CP stromal cells could represent a less mature cell type in the prostate stromal lineage. This is consistent with CP stromal cells showing absent expression of organ-restricted stromal genes [18], lowered expression of genes involved in smooth muscle cell differentiation, and increased expression of the stem cell marker CD90 [4]. Furthermore, we have shown that CP stromal-like cells in gene expression can be generated from NP stromal cells by factors secreted by NCCIT cells in co-culture [8]. In contrast, co-culture with NCCIT showed no significant effect on the gene expression of CP stromal cells. Whole cell extracts from NCCIT had previously been reported to be capable of converting cultured kidney cells into stem-like [32]. As such, CP stromal may be more susceptible to reprogramming than NP stromal. We will test this more rigorously with a number of CP and NP stromal cell populations prepared from the same patients. It is possible that at very high viral titers all cell types could be successfully reprogrammed. In agreement with this supposition is that dental stem/progenitor cells (from exfoliated deciduous teeth, apical papilla, dental pulp) were reprogrammed at a higher rate than fibroblasts [33]. For future clinical application, it is crucial that normal differentiated cell types are more refractory to reprogramming. The similarity in gene expression between the CP stromal-derived iPS cells and human ES cells suggests that these cells could respond to stromal induction by undergoing differentiation as was demonstrated for NCCIT cells [19]. A co-culture of stromal and iPS cells, as was done with NCCIT, is complicated by the use of serum-free TeSR2 media to maintain the stem cells (unlike NCCIT), but stromal cells do not survive well in such media.

Prostate cancer cells could also be viewed as representing a less mature cell type in the epithelial lineage. Compared with luminal cells, the $CD26^+$ cancer cells show lowered expression of many secretory proteins of the prostate [13]. Therefore, one would expect that this cell type to be relatively prone to reprogramming. The technical difficulty concerns whether enough cells can be consistently obtained from tumor samples since the $CD26^+$ primary cancer cells, like terminally differentiated post-mitotic luminal cells, are difficult to being grown in vitro. Nevertheless, the lentiviral vectors are designed to infect efficiently post-mitotic cell types such as neurons [22]. We will test these vectors with adequate amounts of sorted $CD26^+$ cancer cells (10^4–10^5) suspended in media for transfection. If $CD26^+$ cancer cells can indeed be reprogrammed, then a method is at hand that can inactivate the entire gene repertoire of cancer cells including activated oncogenes and mutated genes. The different prostate cancer cell types, as characterized by their transcriptomes, can be grouped into either luminal-like or non-luminal-like/more stem-like [24]. The stem-like grouping contains those that are considered to represent aggressive cancer. Perhaps, these cancer cell types can be more readily reprogrammed than the luminal-like type. Means to enhance the rate of conversion of well-differentiated luminal-like cancer cells may include modulation of certain pathways such as those of p53 and TGFβ [34].

Continuous advances in iPS technology would in the future increase the frequency of conversion, which is currently at 10^{-4} attained with lentiviral vectors. This is comparable to that using human adult fibroblasts [1] or the recently reported 0.01–4% obtained with cells cultured from urine with the lowest frequency from a 65 year donor [35]. Higher percentage, not unexpectedly, could be achieved with less differentiated cell types such as human adipose stem cells obtained from lipoaspiration [36]. iPS cells can also be obtained using chemical reprogramming [37] instead of viral transfection, which has potential untoward side effects. Small molecules have been found that can replace any of the reprogramming TF genes with the added benefit of increasing efficiency and kinetics (i.e., iPS cells in 1 day). Those that act by not involving chromatin remodeling are particularly desirable. These molecules may be administered to patients (e.g., with untreatable disseminated cancer) like drugs. For safety concerns, viral vectors are unsuitable for clinical use. We will test a chemical reprogramming protocol using CP stromal cells once it becomes available.

CONCLUSIONS

CP stromal cells obtained from a patient in his sixth decade can be reprogrammed with four TF genes, NANOG, POU5F1, LIN28, and SOX2, to stem-like with complete inactivation of stromal genes.

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