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Human adipose-derived mesenchymal stromal cell pigment epithelium–derived factor cytotherapy modifies genetic and epigenetic profiles of prostate cancer cells

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Abstract

Background aims—Adipose-derived mesenchymal stromal cells (ASCs) are promising tools for delivery of cytotherapy against cancer. However, ASCs can exert profound effects on biological behavior of tumor cells. Our study aimed to examine the influence of ASCs on gene expression and epigenetic methylation profiles of prostate cancer cells as well as the impact of expressing a therapeutic gene on modifying the interaction between ASCs and prostate cancer cells.

Methods—ASCs were modified by lentiviral transduction to express either green fluorescent protein as a control or pigment epithelium–derived factor (PEDF) as a therapeutic molecule. *PC3* prostate cancer cells were cultured in the presence of ASC culture–conditioned media (CCM), and effects on *PC3* or DU145. *Ras* cells were examined by means of real-time quantitative polymerase chain reaction, EpiTect methyl prostate cancer–focused real-time quantitative polymerase chain reaction arrays, and luciferase reporter assays.

Results—ASCs transduced with lentiviral vectors were able to mediate expression of several tumor-inhibitory genes, some of which correlated with epigenetic methylation changes on cocultured *PC3* prostate cancer cells. When *PC3* cells were cultured with ASC-PEDF CCM, we observed a shift in the balance of gene expression toward tumor inhibition, which suggests that PEDF reduces the potential tumor-promoting activity of unmodified ASCs.

Conclusions—These results suggest that ASC-PEDF CCM can promote reprogramming of tumor cells in a paracrine manner. An improved understanding of genetic and epigenetic events in prostate cancer growth in response to PEDF paracrine therapy would enable a more effective use of ASC-PEDF, with the goal of achieving safer yet more potent anti-tumor effects.

Keywords

adipose-derived mesenchymal stromal cells; epigenetics; gene expression; pigment epithelium—derived factor; prostate cancer

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Introduction

Adipose-derived mesenchymal stromal cells (ASCs) are promising tools for delivery of cancer therapy because they can be obtained with relative ease and are a comparable reservoir of mesenchymal progenitor cells to bone marrow mesenchymal stromal cells (BM-MSCs) (1,2). ASCs are well suited as cellular gene therapy vectors because they can be expanded rapidly, allowing use at low passage numbers, and can be transduced by several viral and nonviral means. ASCs and BM-MSCs share many biological characteristics, including differentiation potential and surface marker expression (1). Interestingly, some evidence has emerged of BM-MSCs ability to exert anti-tumor potential (3–7), a property now also demonstrated for ASC-based therapies. Studies that used ASCs for reducing tumor growth have included the use of wild-type ASCs to inhibit hepatocellular carcinoma, breast and pancreatic tumor growth (8–10) as well as studies examining the potential of ASCs to deliver cytotoxic or secreted therapeutics to tumors. ASCs have been used to deliver tumor necrosis factor ligand member 10 (TRAIL), cytosine deaminase (CD), pigment epithelium–derived factor (PEDF) and melanoma differentiation-associated gene 7 (*MDA-7*), inhibiting growth of several tumor types (11–17).

The use of stem cells to deliver therapeutics remains controversial because the mechanisms underlying the anti-tumor effects are not clear, and, in some cases, stem cells can act to promote tumor growth if used at high concentrations. Therefore, in the present study, our goal was to examine the potential of ASCs to exert either a tumor-promoting or tumor-inhibitory effect in a prostate tumor model system. We examined whether ASCs or ASCs expressing the therapeutic molecule PEDF can modify gene expression and epigenetic methylation profiles of PC3 prostate cancer cells in a coculture system. We selected PEDF because it is downregulated as prostate cancers progress (18), and its loss reduces the ability of prostate cancer cells to respond to inhibitory growth signals. Also, we have shown previously that PEDF is an effective therapeutic against prostate tumors *in vivo* (17). Our results suggest that PEDF modifies the interaction of ASCs with prostate tumor cells, ameliorating and enhancing the intrinsic anti-tumor potential of ASCs, thus comprising a promising therapeutic modality.

Methods

Cell culture, viral transductions and transfections

Human prostate cancer cell lines were obtained from American Type Culture Collection (Manassas, VA, USA) (PC3) or generated by us by means of *Ras* transduction (DU145. *Ras*) as described (19). Human ASCs of low passage numbers (P1–2) were used for viral transduction (17) and then were expanded in culture as described (1) after being isolated from from lipoaspirate tissue donated by three donor patients undergoing elective procedures (with informed consent under a protocol reviewed and approved by the Institutional Review Board, Pennington Biomedical Research Center). Briefly, after ASCs were collected, they were washed three to four times in 1 × Dulbecco's phosphate buffered saline and suspended in 1 × phosphate-buffered saline/1% bovine serum/0.1% collagenase type I (20), with shaking at 37°C for 60 min, spun for 5 min at 300*g* (room temperature) and the pelleted stromal vascular fraction resuspended in Dulbecco's modified Eagle's medium (DMEM)/F12 Ham's/10% fetal bovine serum (FBS)/1% pennicilin/streptomycin, stromal vascular fraction will be plated to 80—90% confluence (∼6 d/∼30,000 cells/cm^2) to yield the initial passage (P0) and seeded at 30,000 cells/cm^2 and cultured to ∼80—90% confluence (∼6 d) for P1—2 to ∼5 × 10^5—10^6. Early-passage (P1—2) ASCs are typically >90%+ CD90/CD105 and <5%+ CD45/CD31 (20) and maintain clonogenic and differentiation capacity (21). Lentiviruses for expressing green fluorescent protein (GFP) or GFP plus human PEDF complementary DNAs were cloned (17) and produced as described

(22). We and others have previously shown that lentivirus gives stable transduction through several passages (17,23); furthermore, transduction efficiency as examined by fluorescence-activated cell sorting for GFP expression was ~60—70%+GFP (unsorted), as is typical for our transduction protocol (24). We used ASC of passage 1—2 (P1—2) to transduce with lentivirus and then used P2—3 after lentiviral transduction for *in vitro* studies. Infection of ASCs was performed in 8 μg/mL of polybrene at lentiviral multiplicity of infection (MOI) of 1. For preparing culture-conditioned media from ASCs (CCM), we plated 5×10^5 ASCs in a six-well format in 2.5 mL/well 10% FBS/DMEM:F12 media (Gibco/Life Technologies, Grand Island, NY, USA). The CCM allowed us to examine the paracrine effects of ASCs (control versus PEDF) on prostate cancer cells. The next day, we changed the media to 2% FBS/DMEM:F12 and collected the resulting ASC CCM 48 h later. This CCM was used to treat PC3 or DU145-*Ras* cells for 48 h for examining the effect of ASC-CM (native, GFP-transduced or GFP-PEDF—transduced) on gene expression or signaling changes compared with PC3 or DU145-*Ras* cells treated with control 2% FBS/DMEM:F12 media (no ASCs).

Clonogenic assay and cell differentiation

For colony formation assay, 250 cells were seeded in a 12-well format and allowed to form colonies over a period of 14d, after which wells were fixed in 10% buffered formalin and stained with the use of 0.5% crystal violet in water. Colonies were counted by means of an NIH ImageJ program. For the differentiation assays, 1.5×10^5 cells were seeded in a 12-well format in triplicate and cultured with differentiation supplements according to the manufacturer's instructions, with the use of Mesenchymal Stem Cell Osteogenesis or Mesenchymal Adipo-genesis Kits (Millipore, Billerica, MA, USA). On day 21 after seeding and culturing in differentiation supplements, cells were stained for either alizarin S red or oil red O stains (Millipore). For soft agar assay, we used a colorimetric Cytoselect cell transformation assay (CellBiolabs Inc, San Diego, CA, USA) in a 96-well format.

Real-time quantitative polymerase chain reaction and EpiTect methyl quantitative real-time polymerase chain reaction array analyses

Total RNA from $5—10 \times 10^5$ cells was extracted with the use of a SurePrep kit (Fisher Scientific). Realtime polymerase chain reaction (RT-PCR) was performed by means of RT^2 SYBR Green ROX qPCR Mastermix (Qiagen, Valencia, CA, USA) with the use of the Realplex 2S cycler (Eppendorf). The PAHS-135Z human prostate cancer—focused quantitative PCR (qPCR) array was used (Qiagen) with 4 μg RNA, according to the manufacturer's protocols. Reactions were carried out by means of 1 × 10 min at 95°C and 40 × 15 seconds at 95°C, 1 min at 60°C. Results for each assay were normalized to the average of all five housekeeping genes. Gene expression changes are reported if a 2.0-fold threshold and a *t* test value of $P < 0.05$ were achieved. For EpiTect methyl qPCR, 1 μg of genomic DNA from PC3 cells was digested according to the manufacturer's instructions. RT-PCR was performed as above, with the use of cycle threshold values to calculate the proportion of hypermethylated (HM), unmethylated (UM) and intermediately methylated (IM) DNA, through the use of the Human Prostate Cancer DNA Methylation PCR Array Signature Panel (EAHS-051Z, Qiagen).

Luciferase assays

Identification of signaling pathway(s) affected by ASC-expressing PEDF and/or rPEDF administration through incubation of prostate cancer cell lines with CCM was performed with the use of Lipofect-amine 2000 (Invitrogen, Carlsbad, CA, USA) transfections in a 96-well format with several plasmid DNAs containing reporter genes downstream of transcription factor—binding elements to help identify the key signaling pathways modulated by treatments. Pathways examined included luciferase-responsive *TGF-β*

(FBEISBE), STAT3, NF-kB (NFkB) and *Elk1* signaling reporters. Plasmids were obtained from Addgene (Cambridge, MA, USA), Clontech or Stratagene (La Jolla, CA, USA). For experiments comparing ASC-PEDF CCM with rPEDF, 288 pg/mL of rPEDF was used in 2% DMEM:F12 media to mimic average PEDF amounts secreted by ASCs into the CCM, as detected by enzyme-linked immunoassay (Figure 1A).

Statistical analysis

Assays were performed in triplicate, and values are provided as mean ± standard error of the mean or 95% confidence intervals. Comparisons were performed by means of an unpaired *t* test, and a value of $P < 0.05$ was considered to indicate a significant difference.

Results

ASC modification with lentiviral vectors

We assessed PEDF expression in the CCM of ASCs *in vitro* after transduction with a pigment-derived epithelial growth factor/GFP therapeutic vector (CMV-PEDF.ires.GFP) as compared with control expressing GFP (CMV-ires.GFP) ($P < 0.001$, Figure 1A). We determined that modification by lentiviral (Mountain View, CA, USA) transduction (MOI =1) did not appear to alter biological characteristics of ASCs examined because both ASC-GFP and ASC-PEDF still were able to form colonies *in vitro* by day 14 of culture (Figure 1A) without any significant difference in colony numbers between ASC types ($P > 0.1$). Additionally, ASCs modified with GFP or PEDF still were able to differentiate into osteogeneic cell lineage as determined by alizarin S red stain (day 21 of culture) as well as the adipogenic lineage, as determined by oil red O stain (day 14 of culture) (Figure 1B).

Several genes are uniquely modulated in *PC3* cells by paracrine ASC mechanisms

We observed several genes with modified expression in *PC3* prostate cancer cells when cultured in the presence of CCM from ASC-GFP (ASCs) or ASC-PEDF (paracrine induction). First, we detailed the gene expression changes reflecting the effect of ASC CCM on *PC3* prostate cancer cells. We used a prostate cancer—focused qPCR assay, which enabled us to query expression changes in 84 genes involved in prostate cancer. The gene expression profiles indicated that ASC coculture induced more prominent upregulation of 10 genes in *PC3* cells as compared with the effect of ASC-PEDF (Figure 2A; $P < 0.01$). These genes included two apoptosis-promoting genes, *CASP3* and cell cycle regulator *CDKN2A* (*pl6*^{INK4a}), three genes downregulated in prostate cancer (*GCA*, *GSTP1*, and *SFRP1*) and one gene encoding a tissue inhibitor of matrix metal-oproteases (*TIMP2*). These changes would be consistent with a potential reduction in PC3 malignancy; however, ASCs also induced four expression changes that could promote malignant potential such as upregulation of a metastatic gene (*CREB1*), upregulation of *CAMSAP1*, a fatty acid metabolism gene typically upregulated in prostate cancer, *PTGS2 (COX2*), an inflammatory gene associated with prostate cancer risk, and *IGFBP5*, a gene upregulated in prostate cancer. Genes downregulated in *PC3* cells on coculture with ASCs included a gene implicated in prostate cancer invasion (*CCNA1*), one gene encoding a transcription factor typically overexpressed in prostate cancer (*EGR3*), a gene overexpressed in tumors including prostate cancer (*GNRH1*) and a fatty acid metabolism gene (*CAMKK1*) (Figure 2B). Although these changes were consistent with a potential reduction in *PC3* malignancy, other changes that could potentially enhance cell proliferation also were observed during incubation with ASC CCM, including down-regulation of tumor suppressor *FOXO1* and transcription factor *MSX1*, both associated with tumor growth inhibition, and downregulation of *PDLIM4*, encoding an actin-binding protein, typically down-regulated during prostate tumorigenesis.

Several genes are uniquely modified in *PC3* cells when cultured in the presence of ASC-PEDF therapeutic CCM

We observed several genes with modified expression in *PC3* cells when cultured in the presence of ASC-PEDF CCM, as compared with control ASC (Figure 2B; $P < 0.01$). These genes included upre-gulation of a tumor-suppressing glutatione peroxidase gene (*GPX3*) and upregulation of *SLC5A8*, a gene typically downregulated in prostate cancer. Also upregulated was *CDH1* (E-cadherin), a change that likely decreases cancer cell invasiveness. These expression changes, probably mediated through paracrine mechanisms by ASC-PEDF, would be consistent with a potential reduction in *PC3* malignancy. However, one gene was upregulated that could promote growth, the fatty acid metabolism gene acetyl-CoA carboxylase-α (*ACACA*). Genes down-regulated in *PC3* on coculture with ASC-PEDF included reductions in two genes typically upregulated in prostate cancer (*KLK3* and *SUPT7L*), several androgen or *PI3KIAkt* signaling genes (*DAXX, IL6, NRIP1* and *VEGFA*). Transcription factors downregulated included *ERG*, whose upregulation is a poor prognosis indicator, *RBM39*, implicated in colorectal carcinoma progression, *SREBF1*, whose inhibition sensitizes cells to death ligands, and one fatty acid metabolism gene (*FASN*) highly upregulated in prostate cancer. Downregulation of the *ETV1* and *MAPK1* genes also should reduce the aggressive phenotype of prostate cancer cells. These expression changes would be consistent with a potential reduction in malignancy of *PC3* cells.

Gene expression changes in *PC3* cells modulated by both ASC and ASC-PEDF CCM

Several gene expression changes were commonly modulated by both ASC types on *PC3* cells, consistent with a reduction in prostate cancer aggressiveness, including upregulation of two genes typically downregulated in prostate cancer (*CLN3, TFPI2*) (Figure 2C; $P < 0.01$). The commonly down-regulated genes modulated by both ASC CCMs on *PC3* included some upregulated in prostate cancer (*DDX11, ECT2, SOX4, CAV2, MTO1*), one metastatic potential gene (*PES1*), genes encoding members of the androgen (*AR, SHBG*) and *P13KIAkt* signaling pathways (*AKT1, BCL2, CCND2*), inflammatory gene *PTGS1 (COX3*) and survival gene *IGF1*. These expression changes might reduce the growth and invasiveness potential of *PC3*. Changes that would be consistent with enhancing *PC3* cell growth by both ASCs could include downregulation of genes already downregulated in prostate cancer (*PPP2R1B*), transcription factor (*SOX4*), and downregulation of a tumor suppressor hyper-methylated in prostate cancer (*RARB*). Additional changes consistent with growth promotion might include two genes encoding for enhanced metastatic potential (*MAX, NDRG3*), a gene belonging to *PBKIAKT* signaling (*PDPK1*), a gene upregulated in prostate cancer (*ARNTL*) and two genes involved in fatty acid metabolism (*PRKAB1, STK11*).

Methylation changes are detectable in *PC3* cells in response to ASC or ASC-PEDF CCM

We examined methylation changes in focused prostate cancer—related genes with the use of the EpiTect Methyl II PCR array (Qiagen), which allowed profiling of HM, UM or IM epigenotypes. The epi-genotypes were examined simultaneously for a panel of 22 gene promoters selected on the basis of their reported methylation status in prostate cancer progression. We observed several changes when *PC3* cells were cocultured with either ASC or ASC-PEDF (Figure 3). The coculture with ASCs induced detectable upregulation of several genes in *PC3* cells, including *CDKN2A, DKK3, PTGS2, SFRP1* and *TIMP2* in HM (Figure 3A, yellow) and UM (Figure 3B, blue) methylation profile subsets. The UM profile displayed additional changes undetected in the HM subset, including upregulation of *CDH1, GSTP1, MSX1, RASSF1* and *SLC5A8* (Figure 3B, blue). ASCs induced detectable upregulation of *GPX3* and downregulation of several genes in the *PC3* HM profile including *AR, MGMT* and *RARB* (Figure 3A, blue). ASCs induced detectable downregulation also of

APC, *EDNRB*, *PDLIM4* and *TNFRSF10D* in the UM methylation subset (Figure 3B, yellow). A third methylation profile that we also examined was the IM group, in which *CDH1*, *GSTP1* and *MSX1* were upregulated as an effect of ASC on *PC3* cells (Figure 3C, yellow). The culture with ASC-PEDF CCM induced detectable upregulation of several genes in *PC3* prostate cancer cells, including *DKK3*, *DLC1*, *GPX3*, *RASSF1*, *SFRP1* and *ZNF185*, as detected in both HM (Figure 3D, yellow) and UM (Figure 3E, blue) methylation profile subsets. The HM subset also presented upregulation of *GSTP1*, *MGMT* and *TIMP2* (Figure 3D, yellow), whereas the UM also presented upregulation of *APC*, *CDH1*, *SLC5A8*, *TNFRSF10D* and *MSX1* (Figure 3E, blue). ASC-PEDF may induce downregulation of *AR*, *CDH1* and *RARB* as detected in the HM subset (Figure 3D, blue). The IM subset suggested that ASC-PEDF may induce upregulation of *CDH1*, *MSX1* and *ZNF185* (Figure 3F, yellow).

Native ASCs and lentiviral-transduced ASCs: similarities and differences

We examined whether lentiviral transduction of ASCs, albeit at a low MOI (= 1), could alter ASC paracrine interactions with prostate cancer cells *PC3* or DU145.*Ras*. We selected 27 genes from the gene expression and methylation profiles observed in *PC3* cells for the next analyses. Fifteen of these 27 genes were further validated by qPCR in *PC3* cells in independent experiments to test the gene expression effect of native, untransduced ASC (ASCn) native versus ASC-GFP (ASCg). We observed that ASCn CCM mediated upregulation of *VEGF*, *SOCS3* and *PDLIM4* in both prostate cancer cell lines, upregulation of *CAV1* and downregulation of *TNFRSF10d* in PC3 cells. ASCg mediated upregulation of *DKK3*, *SLCA58*, *SOCS3* and *CAV1* in *PC3* cells and upregulation of *VEGF* and downregulation of *ERG*, *CAV1* and *EDNRB* in DU145.*Ras* cells (Figure 4A). We also examined several signaling pathways potentially affected in prostate cancer cell lines on exposure to ASC CCM. Similar effects included upregulation of *NFkB* signaling by ASCg in both prostate cancer cell lines and by ASCn on DU145.*Ras* (Figure 5B). Also, ASCn and ASCg both upregulated *STAT3* signaling and down-regulated *Elk1* signaling in DU145.*Ras*.

ASC-PEDF and rPEDF therapies

We examined the effects of PEDF derived from ASC CCM and recombinant PEDF to determine whether there is a specific effect when PEDF is delivered from ASCs in the CCM (paracrine manner). We used the same gene panel from Figure 4 to examine the gene expression profiles modulated by ASC-PEDF versus rPEDF on PC3 or DU145.*Ras* prostate cancer cells (Figure 5A). In *PC3* cells, ASC-PEDF upregulated *SOCS3* at a higher magnitude (6-fold) than did rPEDF or controls. Unique changes modulated by rPEDF were downregulation of *IL6* and *TNFRSFlOd* genes in *PC3* cells. In DU145.*Ras* cells, ASC-PEDF uniquely upregulated *VEGF*, *SLCA58* and *SOCS3*, whereas rPEDF uniquely downregulated *SCAF11*, *IL6*, *VEGF*, *DKK3*, *SLCA58*, *PDLIM4*, *SOCS3* and *EDNRB*. We tested the whole panel of luciferase reporters from Figure 4B; however, only one to two signaling pathways showed showed significant changes in each prostate cancer cell line. For *PC3*, both ASC-PEDF CCM and rPEDF showed upregulation of TGF-β signaling ($P < 0.01$), although ASC-PEDF modulated a significantly higher TGF-β signaling upregulation compared with rPEDF (Figure 5B; $P < 0.03$). In PC3, ASC-PEDF CCM (but not rPEDF) downregulated *STAT3* signaling. In DU145.*Ras*, both ASC-PEDF CCM and rPEDF induced reduced *STAT3* signaling in prostate cancer cells (Figure 5B, $P < 0.02$), and the effect of ASC-PEDF was greater than that of rPEDF ($P < 0.04$). In both cell lines, the effect of PEDF was confirmed by use of a specific antibody, anti-PEDF, which at least partially reversed the effects of PEDF on signaling on both cancer cell lines as compared with isotype control (Figure 5B).

Discussion

The use of a cellular carrier can be enhanced when secreted gene therapy molecules are used, and we have shown that ASCs are suitable vehicles for delivery of PEDF to treat prostate tumors (17,25). Our previous work has shown that ASCs are amenable to stable or transient genetic modification, enabling delivery of secreted therapeutic molecules into the culture-conditioned media without adverse effects on ASC viability or differentiation. One major consideration for potentially translating ASC therapies is that they are non-tumorigenic once introduced into the recipient. Although we have not found ASC to be tumorigenic (17,25), this subject still is controversial. Other reports either support (11,15,26) or oppose our findings (12,27,28). Interestingly, some recent studies proposed that BM-MSCs or ASCs might have a potent tumor-inhibitory effect, acting to inhibit tumor growth, as reported for pancreatic models through induction of apoptosis (8), in hepatocellular carcinoma and in Kaposi sarcoma models through down-regulation of *AKT* signaling (10,29) and in colorectal cancer through induction of pro-apoptotic genes (30). Therefore, our present study was designed to examine gene expression, epigenetic profiles, and signaling pathways modified in prostate cancer cells on culture with ASC CCM. Our goal was to gain insight into whether ASCs (and/or therapeutically modified ASC-PEDF cells) are capable of inducing a profile balanced toward tumor-inhibition in a paracrine manner.

We observed several interesting paracrine effects on *PC3* prostate tumor cells on incubation with ASC CCM that would be consistent with a tumor-inhibitory effect of ASC, including upregulation of apoptosis/cell cycle regulators *CASP3* and $pl6^{INK4a}$ and reductions in metastasis/invasion gene *CCNA1*. Also, genes typically downregulated in prostate cancer were activated (*GCA*, *SFRP1*), along with an inhibitor of MMPs (*TIMP2*). Other expression reductions included two genes overexpressed in prostate cancer (*EGR3* and *GNRH1*) and *CAMKK1*, a gene involved in fatty acid metabolism. ASC CCM also induced some changes that could promote prostate tumors such as upregulation of metastasis/growth gene *CREB1* and downregulation of tumor suppressors *FOXO1* and *MSX1*, and the actin-binding protein *PDLIM4*, all associated with tumor growth inhibition. Genes usually upregulated in prostate cancer were further upregulated, including *CAMSAP1*, *PTGS2* and *IGFBP5*. Several of these gene expression changes correlated with altered methylation profiles as well, which suggests that regulation occurs at least in part through epigenetic interactions.

ASC-PEDF CCM induced a much more clear anti-tumor profile of gene expression changes in *PC3* cells, with downregulation of four *ARIPI3KIAKT* signaling genes (including *IL6*), whereas four genes usually downregulated in prostate cancer or associated with a poor prognosis were upregulated. Furthermore, four genes typically upregulated in prostate cancer (*KLK3* and *SUPT7L*) or associated with aggressiveness (*ETV1*, *MAPK1*) were downregulated. Despite a majority (∼93% of changes observed) of gene expression changes being consistent with tumor-inhibitory mechanisms by ASC-PEDF CCM, three gene expression changes observed still could potentially promote prostate tumor growth, including enhanced expression of the fatty acid metabolism gene *ACACA*, upregulation of *SLC5A8*, a gene typically downregulated in prostate cancer, as well as further downregulation of *CAV1*, typically downregulated in prostate cancer (*CAV1*). Given this overall gene expression response, ASC-PEDF CCM might be promoting regulatory changes at the level of the Gl/S transition (10) or could be stimulating expression of anabolic enzymes to coordinately regulate energy production and biosynthesis at multiple levels.

Common expression changes modulated by both ASC and ASC-PEDF include 15 tumor-inhibitory and 9 tumor-promoting genes. Of note are tumor-inhibitory changes mediated by both ASC or ASC-PEDF, including reductions in *PI3KIAKT* and *AR* signaling and inflammatory or survival pathways (*COX3*, *IGF1*), and reduced expression of the metastasis

gene *PES1*. Of the potential tumor-promoting changes, metastasis and fatty acid metabolism genes were upregulated, and genes usually downregulated in prostate cancer were further reduced; one (*RARB*) correlated with methylation profiling. Also, genes usually upregulated in prostate cancer were upregulated such as *ARNTL*. Another ASC-PEDF—specific anti-tumorigenic expression pattern that correlated with methylation status was upregulation of *GPX3*, a proapoptosis/tumor suppressor gene. *GPX3* (glutathione peroxidase 3) is a selenium-dependent enzyme that plays a critical role in detoxifying reactive oxidative species and maintaining the genetic integrity of mammalian cells. *GPX3* is widely inactivated in prostate cancers and has been shown to be a potent tumor suppressor. *PC3* xenografts expressing *GPX3* show a reduction in tumor volume by ∼ 5-fold, elimination of metastasis and improved survival (31). This suggests that ASC-PEDF CCM could mediate the paracrine antitumor effects *in vivo* through epigenetic reactivation of *GPX3*. Additional gene expression changes mediated on *PC3* included *SOCS3* upregulation in *PC3* cells with a higher magnitude (∼ 6-fold) by ASC-PEDF CCM as compared with rPEDF or controls. A unique change modulated by rPEDF included downregulation of *IL6* in *PC3* and DU145. *Ras* cells and downregulation of *SOCS3* in DU145. *Ras* cells. These changes, in combination with reporter gene activity data, suggest that *SOCS3* upregulation may render the ASC-PEDF CCM therapy more effective in its suppression *of STAT3* activity as compared with rPEDF. *SOCS3* is a negative regulator of the *IL6ISTAT3* signaling axis (32) and can be subject to reactivation from epigenetic silencing; future studies will examine this interesting hypothesis. Reactivation could be a plausible explanation for why, even though *IL6* message levels are low, *SOCS3* levels are elevated, enabling *SOCS3* to inhibit *JAK* activity and thus reduce *STAT3* signaling in prostate cancer cells treated with ASC-PEDF CCM. The mechanisms of anti-tumor activity of ASC-PEDF CCM, however, appear to be complex and distinct for different prostate tumor cell lines. For example, in DU145-*Ras*, the effects of ASC-PEDF CCM included only *STAT3* down-regulation, whereas in *PC3* cells, the effect combined *STAT3* downregulation and TGF-β signaling upregulation.

We also examined whether lentiviral transduction of ASCs could alter their ability to interact with prostate cancer cells in a paracrine manner. Overall, it appeared that both native ASCs (untransduced, ASCn) and ASC-GFP (ASCg) CCM could upregulate protumorigenic factors *VEGF* and *ERG* while upregulating the anti-tumor gene *SOCS3*. Also, even though ASCn upregulated *PDLIM4*, ASCg upregulated *DKK3*, *SLCA58*, *CAV1* and *EDNRB*, and all of these are anti-tumor changes that may serve to regulate prostate cancer growth. The signaling pathways examined by reporter gene expression included upregulation of *NFkB* and *STAT3* and down-regulation of *Elk 1* signaling by both ASCn and ASCg in prostate cancer cells. These results suggest that lentiviral transduction might alter how ASCs mediate expression of tumor-promoting genes in tumor cells, but the expression and reporter gene activity profiles were considered to be generally equivalent.

Overall, this study is particularly interesting because in our previous work, we did not detect any tumorigenic potential for either ASC-GFP or ASC-PEDF *in vivo* (17). However, in the present (*in vitro*) study, some potential protumorigenic changes are detected in gene expression profiles of prostate cancer cells when incubated with ASC CCM. This apparent discrepancy would suggest that *in vivo*, the genetic and epigenetic changes presented here tilt the balance toward apoptosis and growth inhibition of prostate cancer cells. The relationship between the mediators identified here may be related to *GPX3* through its ability to control the release of inflammatory mediators/adipokines through reduced reactive oxygen species and DNA damage (33,34). The potential *GPX3* reactivation by ASC-PEDF CCM might act as a tumor suppressor to modify *IL6* or other inflammatory cytokine signals as well as other adipokine (*TGF-β*) signals to affect tumorigenesis. Functioning as a transcription factor, *STAT3* participates in the signaling pathways for many cytokines in various cells and organs that are regulated by the suppressor of cytokine signaling family,

including *SOCS3*. *STAT3* and *SOCS3* can be potential targets for regulating inflammatory responses. Another signaling pathway of interest may include *TGF-β*, which might under some conditions act as a growth-inhibitory signal. Studies that used MSC CCM for treating experimental colitis support this hypothesis (35). MSCs upregulated *VEGF* and *MCP1* but also modulated molecules related to the *TGF-β* signaling pathway (upregulated activin A, *TGF-β* and down-regulated inhibin bA). MSC CCM also mediated paracrine changes in *RAW264.1* cells and reduced secretion of proinflammatory *TNF* and *IL6* cytokines in the same colitis model. Another interesting difference between studies is that *in vivo*, ASCs secreting PEDF engraft into the tumor, and this action might maximize their therapeutic effect compared with *in vitro* studies with CCM. The colitis model cited above would support this hypothesis because it reported that CCM therapy required a 9-fold increase in the number of MSCs compared with cellular therapy (35).

Conclusions

An interesting aspect that we have thus observed for ASC or ASC-PEDF therapeutics is their potential ability to alter gene expression and to potentially reverse certain epigenetic marks with relevance to prostate cancer. A better understanding of the linkage between genetic and epigenetic events in tumor growth probably will result in a more effective use of ASC or ASC-PEDF for treating prostate cancer. We anticipate that delivery of immunostimulatory cytokines (36), anti-angiogenic molecules, apoptosis-inducing genes, nanoparticles and/or therapeutic exosomes (37) by ASCs or other stem cells to tumors will comprise very promising approaches for antitumor therapy. Future successful therapies may combine traditional targeted and stem cell—directed gene therapies to enable significant advances in the treatment of prostate and other cancers.

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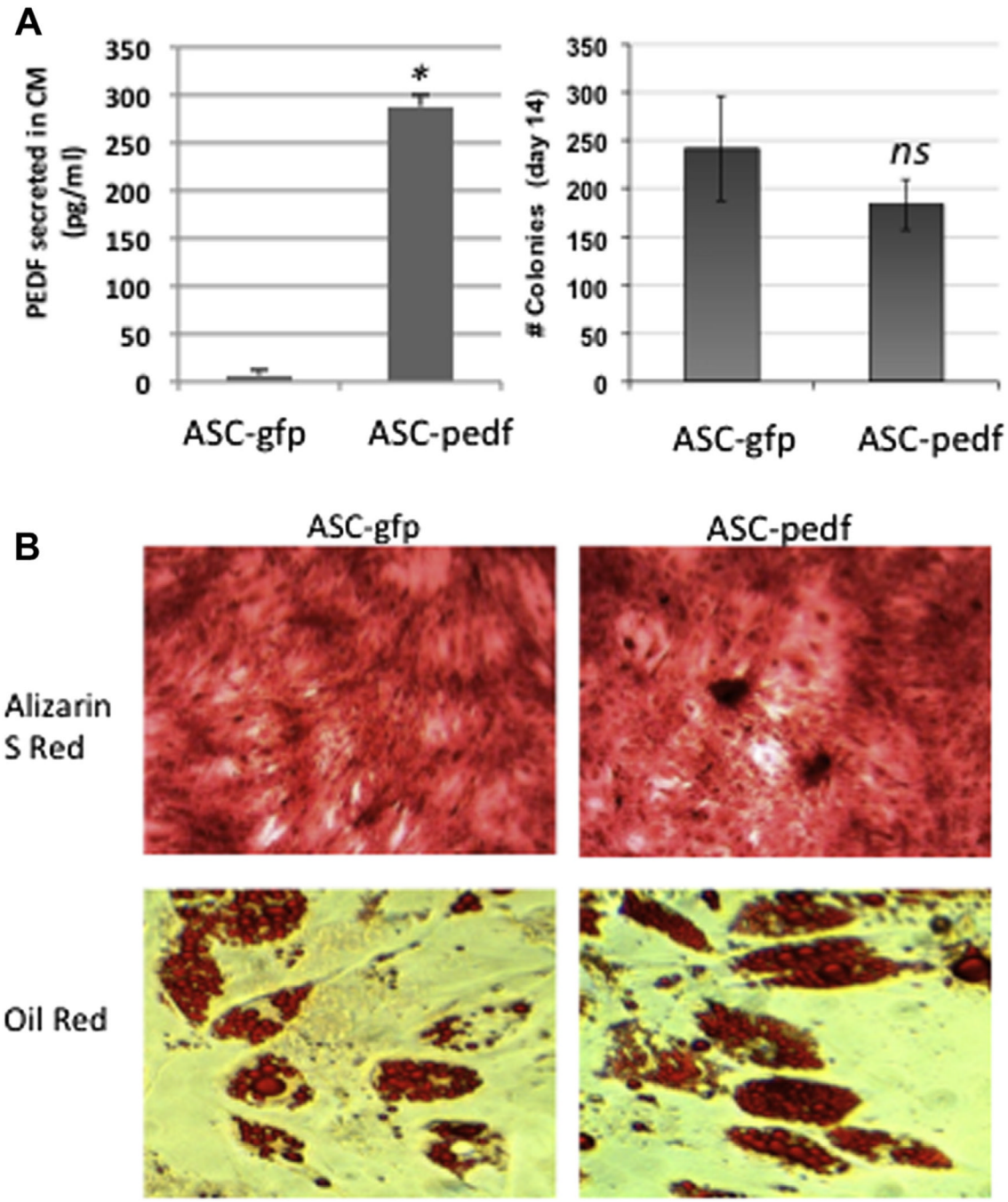

Figure 1.

Biological characteristics of ASCs modified with lentiviruses. (A) ASCs modified with either control lentivirus (CMV-ires.GFP; ASC-GFP) or CMV-PEDF.ires.GFP lentivirus (ASC-PEDF) at MOI = 1 still form equivalent numbers of clones in a clonogenic assay (P = 0.1). (B) Differentiation capacity does not appear to be impaired by transduction with PEDF as assessed by ability to differentiate into osteogenic cell lineage (alizarin S red stain) or adipogenic cell lineage (oil red O).

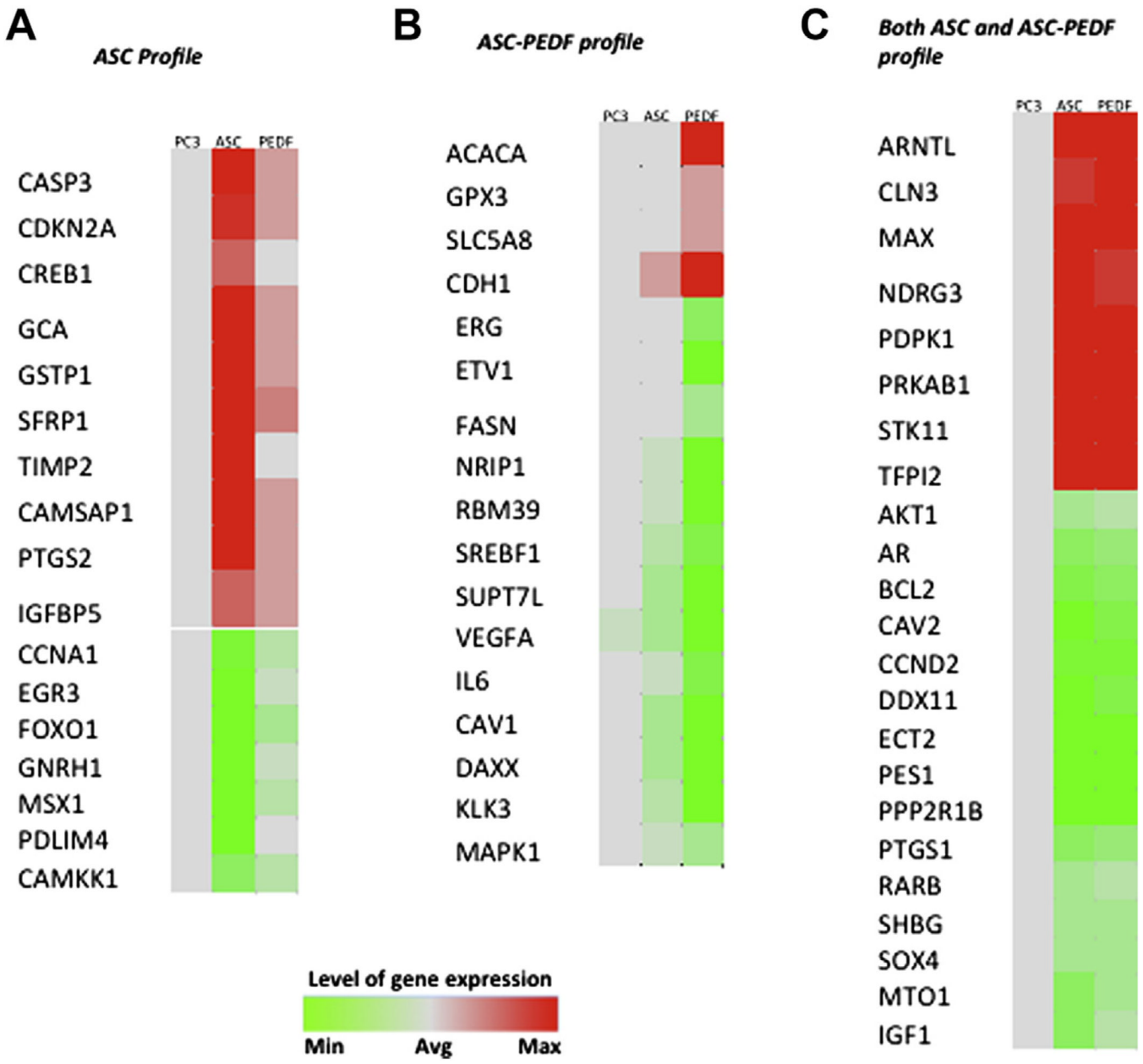

Figure 2.

Heat map shows paracrine effect of ASCs or ASC-PEDF on *PC3* gene expression changes. (A) Gene changes effected on *PC3* cells by ASCs. (B) Gene changes effected on *PC3* cells by ASC-PEDF. (C) Gene changes effected on *PC3* cells both by ASC and ASC-PEDF. Color bar indicates the level of gene expression for each gene depicted ranging from green (minimum) to red (maximum) (cutoff of $P < 0.01$ for gene expression changes).

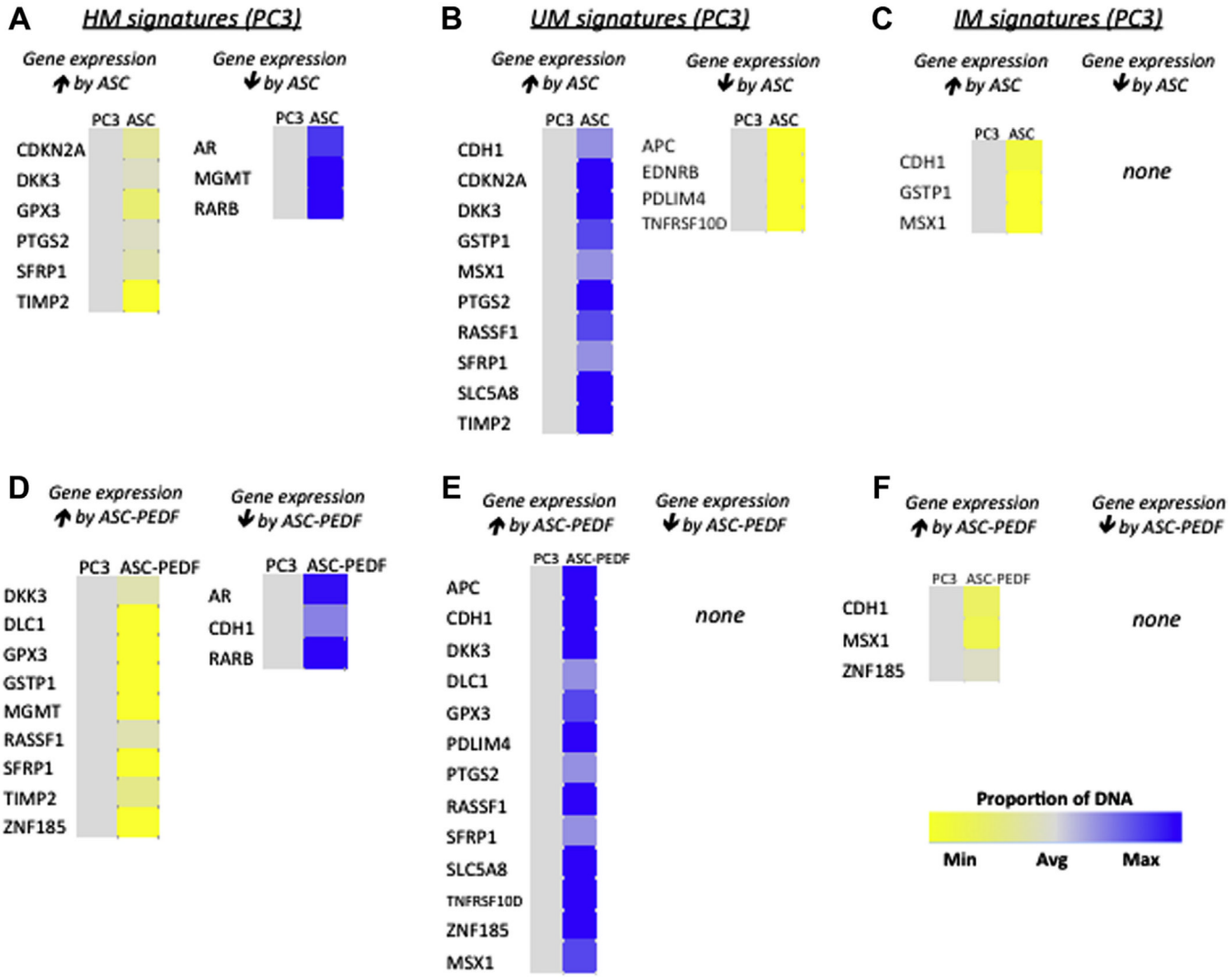

Figure 3.

Heat map shows paracrine effects of ASC or ASC-PEDF on *PC3* methhylation profiles. HM signatures detected in *PC3* cells after coculture, with the predictions of which changes might reduce or enhance gene expression by ASC (A) or ASC-PEDF (D). UM signatures detected in *PC3* cells after coculture, with the predictions of which changes might reduce or enhance gene expression by ASC (B) or ASC-PEDF (E). IM signatures detected in *PC3* cells after coculture, with the predictions of which changes might reduce or enhance gene expression by ASC (C) or ASC-PEDF (F). Color bar indicates the proportion of DNA detected in each category for each gene depicted ranging from yellow (minimum) to blue (maximum) (cutoff of $P < 0.01$ for gene expression changes).

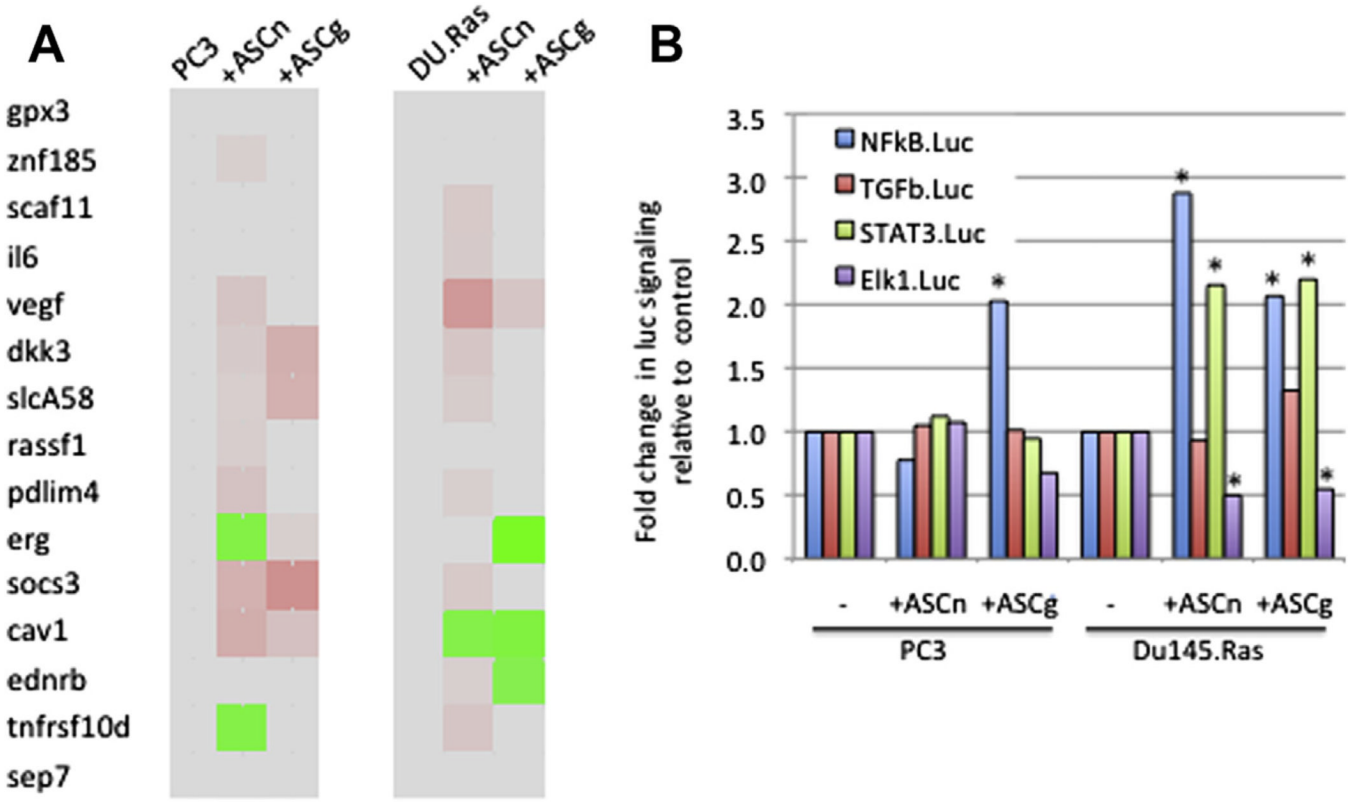

Figure 4.

Comparison between ASC native and GFP-transduced ASC (AS-GFP). (A) Gene expression profiles for *PC3* or DU145-*Ras* cells treated for 48 h with either control CCM (no ASC), CCM from ASC-native (ASCn) or ASC transduced with MOI = 1 of Lv-GFP (ASCg). (B) Luciferase reporter assays for *PC3* or DU145-*Ras* cells treated as in (A). **P* < 0.01.

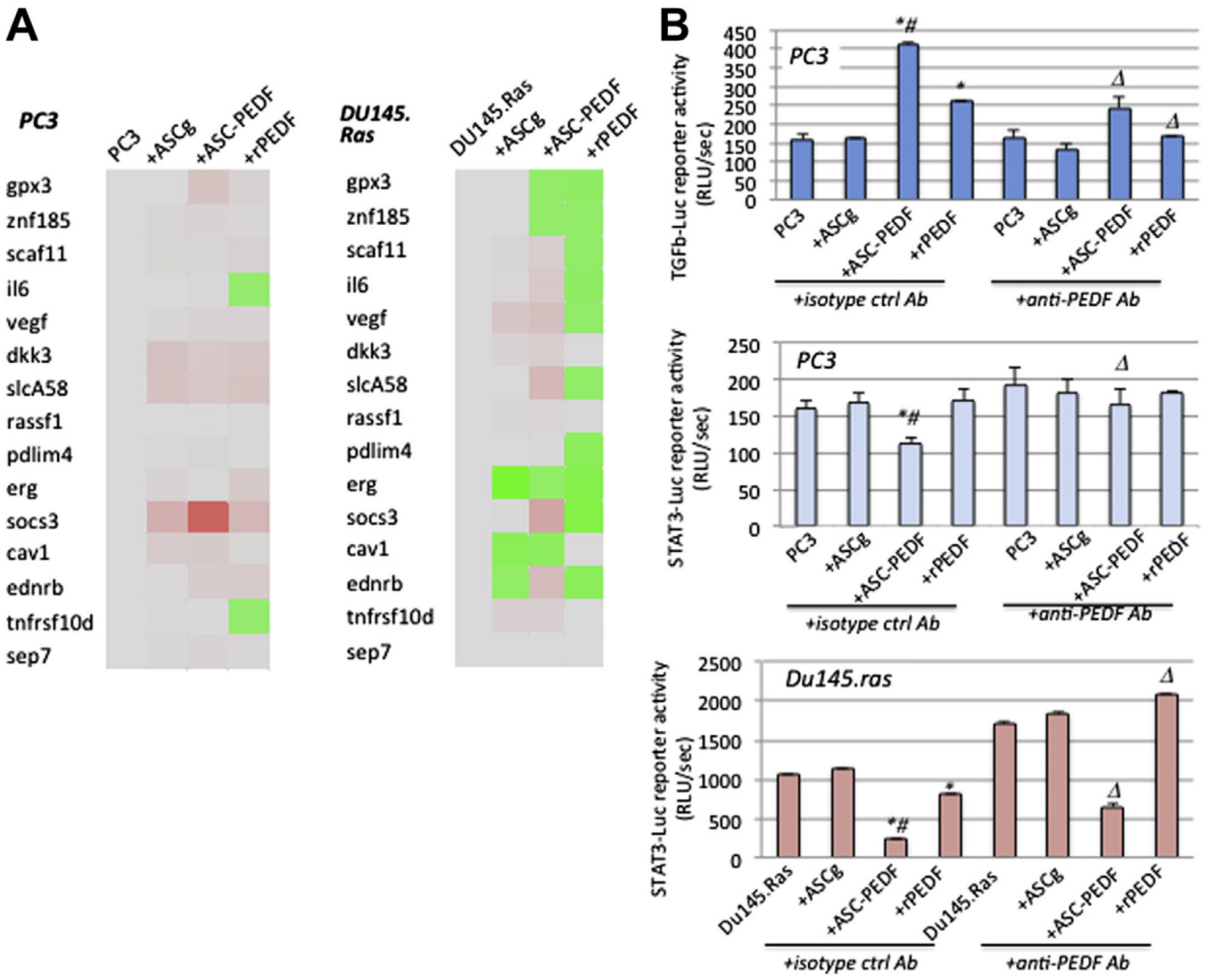

Figure 5.

Comparison between PEDF therapy derived from ASC-PEDF CCM and recombinant PEDF. (A) Gene expression profiles for *PC3* or DU145-*Ras* cells with either control CCM (no ASC), CCM from ASC-GFP (MOI = 1), ASC-PEDF-GFP (ASC-PEDF, MOI = 1) or recombinant PEDF (rPEDF) as described in the Methods section. (B) Select luciferase reporter assays for *PC3 (TGF-β-luc)* or DU145-*Ras (STAT3-luc)* cells treated as in (A). $^{*}P < 0.05$ compared with controls (prostate cancer cells alone or ASCg); $^{\#}P < 0.05$ for ASC-PEDF compared with rPEDF and $\Delta P < 0.05$ for anti-PEDF antibody effect as compared with isotype control-treated.