J. Cell. Mol. Med. Vol 20, No 4, 2016 pp. 721-730

Protein profile of basal prostate epithelial progenitor cells—stage-specific embryonal antigen 4 expressing cells have enhanced regenerative potential *in vivo*

Thomas Höfner [a, b, *], Corinna Klein [a], Christian Eisen [a], Teresa Rigo-Watermeier [a], Axel Haferkamp [b], Martin R. Sprick [a, c, d, *]

[a] *Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Heidelberg, Germany*
[b] *Department of Urology, University Hospital Frankfurt, Frankfurt, Germany*
[c] *Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany*
[d] *German Cancer Consortium (DKTK), Heidelberg, Germany*

Received: August 15, 2015; Accepted: December 13, 2015

Abstract

The long-term propagation of basal prostate progenitor cells *ex vivo* has been very difficult in the past. The development of novel methods to expand prostate progenitor cells *in vitro* allows determining their cell surface phenotype in greater detail. Mouse (Lin^- $Sca\text{-}1^+$ $CD49f^+$ $Trop2^{high}$-phenotype) and human (Lin^- $CD49f^+$ $TROP2^{high}$) basal prostate progenitor cells were expanded *in vitro*. Human and mouse cells were screened using 242 anti-human or 176 antimouse monoclonal antibodies recognizing the cell surface protein profile. Quantitative expression was evaluated at the single-cell level using flow cytometry. Differentially expressed cell surface proteins were evaluated in conjunction with the known $CD49f^+/TROP2^{high}$ phenotype of basal prostate progenitor cells and characterized by *in vivo* sandwich-transplantation experiments using nude mice. The phenotype of basal prostate progenitor cells was determined as $CD9^+/CD24^+/CD29^+/CD44^+/CD47^+/CD49f^+/CD104^+/CD147^+/CD326^+/Trop2^{high}$ of mouse as well as human origin. Our analysis revealed several proteins, such as CD13, Syndecan-1 and stage-specific embryonal antigens (SSEAs), as being differentially expressed on murine and human $CD49f^+$ $TROP2^+$ basal prostate progenitor cells. Transplantation experiments suggest that $CD49f^+$ $TROP2^{high}$ $SSEA\text{-}4^{high}$ human prostate basal progenitor cells to be more potent to regenerate prostate tubules *in vivo* as compared with $CD49f^+$ $TROP2^{high}$ or $CD49f^+$ $TROP2^{high}$ $SSEA\text{-}4^{low}$ cells. Determination of the cell surface protein profile of functionally defined murine and human basal prostate progenitor cells reveals differentially expressed proteins that may change the potency and regenerative function of epithelial progenitor cells within the prostate. SSEA-4 is a candidate cell surface marker that putatively enables a more accurate identification of the basal PESC lineage.

Keywords: CD13 • Syndecan-1 • prostate stem cells • prostate progenitor cells • SSEA

Introduction

Several model systems have been developed to understand the biological mechanisms involved during benign prostatic enlargement and prostate cancer, the latter being the most common type of cancer in men. It has been suggested that basal epithelial stem/progenitor cells (basal PESCs) are critical for the development of the prostatic gland and that they play an important role in prostate cancer development [1–4]. However, basal PESCs are rare, with a frequency of 1–5% within all prostatic cells, which clearly complicates biological studies using these cells [5, 6]. Isolation and *ex vivo* expansion of basal PESCs have been further complicated by their dependence on poorly understood factors supplied by a prostate cell niche composed of smooth muscle cells, fibroblasts, neuroendocrine cells, and differentiating and mature prostate epithelial cells [7]. Although significant progress had been made, culture techniques up to now allowed for only limited expansion of prostate epithelial cells (PrECs), which rapidly ceased to proliferate [8–10]. We recently discovered new methods to grow and expand both murine and human basal PESCs in serum- and feeder-free conditions [11]. The methods enrich for

*Correspondence to: Thomas HÖFNER and Martin R. SPRICK
E-mails: thomas.hoefner@kgu.de and martin.sprick@hi-stem.de

doi: 10.1111/jcmm.12785

adherent mouse basal PESCs with a Lin^- $Sca\text{-}1^+$ $CD49f^+$ $Trop2^{high}$ phenotype. Progesterone and sodium selenite are additionally required for the growth of human Lin^- $CD49f^+$ $TROP2^{high}$ basal PESCs. When transplanted in combination with urogenital sinus mesenchyme (UGSM), expanded mouse and human basal PESCs generate ectopic prostatic tubules, demonstrating their stem cell activity *in vivo* [11]. The possible expansion of basal PESCs to significant cell numbers allowed us high-throughput analyses to characterize their cell surface protein profile in detail.

Materials and methods

Adherent expansion of primary murine and human basal PESCs

Murine and human basal PESCs were isolated and propagated as described [11]. Microdissection, enzymatic digestion and preparation of single cells from male C57Bl/6 mice were performed as described previously [6]. For isolation of primary human cells from surgical prostate tissues, we obtained informed consent according to the principles of the Declaration of Helsinki. Procedures were approved by the responsible ethics committee of Heidelberg University (permit S-479/2009). Briefly, MACS enrichment for EPCAM+ cells was performed after primary preparation of single-cell suspensions from murine and human prostates. Magnetic enrichment was performed using the autoMACS Pro Separator (Miltenyi Biotec, Bergisch-Gladbach, Germany). After magnetic enrichment, murine cells were cultured (adherent) on hydrophobic (suspension) culture flasks (Cellstar; Greiner Bio-One, Kremsmuenster, Austria), and human cells were cultured on net-negative pretreated surface flasks (Primaria; BD, Franklin Lakes, New Jersey, USA). The best media for the expansion of murine basal PESCs consists of Advanced DMEM/F12 supplemented with additional glutamine, glucose, EGF, bFGF, LONG R3 IGF-I, holo-transferrin and insulin. The best media for the expansion of human basal PESCs is the murine formulation plus additional progesterone and sodium selenite. We previously characterized expanded basal PESCs demonstrating predominant basal cell marker (*e.g.* CK5, p63) expressions within the undifferentiated progenitor culture [11].

High-throughput screening of cell surface proteins by flow cytometry

We prepared single-cell suspensions of 1.5×10^8 cells from each murine and human culture cells using StemPro-Accutase (Gibco, Carlsbad, California, USA) corresponding to culture passage 7 and 8. Cells were stained in 96-well plates using BD Lyoplate Cell Surface Marker Screening Panels containing antibodies against 242 human or 176 mouse cell surface antigens, respectively. These panels include the appropriate isotype controls for each antibody used. After washing, cells were resuspended in stain buffer [PBS supplemented with 5 mM ethylenediaminetetraacetic acid (EDTA)] containing propidium iodide for dead cell exclusion. Flow cytometry screening (Alexa 647 signals) was performed using a BD FACS Array Bioanalyzer system, and final analysis was done with FlowJo software (Tree Star, Inc, Ashland, Oregon, USA).

Sorting and mouse experiments to evaluate *in vivo* stem cell capability

All cell sortings were performed on BD FACS Aria II cell sorter using a 100 μM nozzle. To minimize loss of cell viability, we performed experiments on cell suspensions, prepared shortly before flow cytometry from cultured cells. We detached the cells using StemPro-Accutase (Gibco). Antibody staining was performed in PBS supplemented with 5 mM EDTA. Prior to flow cytometry or sorting, cells were filtered using 40-μm filters. The sorting buffer included PBS, 5 mM EDTA and 10 mM ROCK inhibitor (Y-27632; Tocris Bioscience, Tocris, Bristol, UK). Forward-scatter height (FSC-H) *versus* forward-scatter width (FSC-W) and side-scatter height (SSC-H) *versus* side-scatter width (SSC-W) profiles were used to eliminate cell doublets. Dead cells were eliminated by excluding PI+ cells, whereas contaminating human or mouse Lin+ cells were eliminated by gating on Ter119/CD31/CD45-FITC for mouse and CD45/CD3-FITC for human cells. Gates for FACS experiments were determined by using isotype controls for the respective specific antibodies used. Gates were then set to exclude the respective population in the isotype control experiment. All mouse experiments were approved by the animal-protection officers of the German Cancer Research Center (DKFZ) and in accordance with German law (Approval number, G18-12). Male nude mice were bred at the animal facility of the DKFZ and maintained under pathogen-free, individual ventilated-cage conditions. E16 UGSM was used for coinjections with culture-derived basal PESCs to provide the necessary growth signals to promote *in vivo* prostate gland regeneration. Before performing the coinjections, UGSM was prepared freshly from foetuses of E16 C57Bl/6 mice as previously described by Lukacs *et al.* [6]. Briefly, timed pregnancies of C57/BL6 mice were set up and pregnant females were killed at day 16 (E16). Under the stereomicroscope, the foetus was cut in half, the bottom half placed in a supine position while holding the hind legs apart. The urogenital sinus is connected to the bladder and was removed intact, followed by enzymatic digest of the microdissected mesenchyme with 10× collagenase for 2 hrs. Urogenital sinus mesenchyme cells were washed in PBS (4°C) and filtered with 40 μm pore size before used in coinjections with basal PESCs [6].

Proof of *in vivo* prostate regeneration by lentiviral gene transfer in expanded PESCs

The LeGO-V2 (Venus) vector was previously described [12] and kindly provided by Kristoffer Weber and Boris Fehse. Lentiviral particles were generated as previously described [13]. For transduction, human basal PESCs were cultured for 24 hrs at a fixed cell number. Target cells were incubated in the presence of 8 μg/ml polybrene for 12 hrs at 37°C with viral supernatant at a multiplicity of infection of 50–60 per vector. Transduction efficiency was validated 48–72 hrs after transduction using FACS. To prove *in vivo* stem cell capability of our culture-derived cells, we coinjected LeGO-V2 marked cultured human basal PESCs together with E16 UGSM and Matrigel into male nude mice subcutaneously. To support differentiation, we subcutaneously implanted testosterone pellets (12.5 mg/90-day release; Innovative Research of America). After 10–12 weeks, we harvested the regenerated s.c. grafts for subsequent analyses. Before conducting histological analyses on fixed tissue, we validated direct Venus fluorescence in freshly dissected s.c. grafts under the fluorescence stereomicroscope. Detection of Venus+ in regenerated prostate tissue (proof of regeneration from

transplanted PESCs origin) was done by staining s.c. grafts with a monoclonal antibody against GFP/Venus (ab 290; Abcam, Cambridge, UK) [11].

Statistical analysis

All data are presented as mean ± S.E.M., comparison between groups was done using non-parametric Kruskal–Wallis tests. (Graph Pad Prism 5.04, Graph Pad Software, La Jolla, California, USA) was used for statistical analyses.

Results

High-throughput screen identifies the specific cell surface protein profile of murine basal PESCs

Using the recently established method, we could expand murine Sca-1$^+$/CD49f$^+$/Trop2^{high} basal PESCs up to 1.5 × 10^8 cells and investigated their cell surface protein profile using 176 validated monoclonal antibodies (results in Table 1).

High-throughput screen identifies the specific cell surface protein profile of human basal PESCs

We next performed this screen with *ex vivo* expanded human CD49f$^+$/TROP2^{high} basal PESCs using 242 validated monoclonal antibodies (Table 2). Staining of murine and human cells revealed that basal PESCs, in addition to their expression CD49f$^+$/TROP2^{high}, are positive for a variety of additional markers (Tables 1 and 2). Both mouse and human basal PESCs share the CD9$^+$/CD24$^+$/CD29$^+$/CD44$^+$/CD47$^+$/CD49f$^+$/CD104$^+$/CD147$^+$/CD326$^+$/Trop2^{high} lineage. Table 3 illustrates the cell surface proteins that are well conserved or differentially expressed among both species.

Cell surface protein screen identifies CD13 and Syndecan-1 as being heterogeneously expressed in CD49f$^+$/TROP2^{high} human basal PESC

By using the cell surface screen, we identified proteins that were differentially expressed within the CD49f$^+$/TROP2^{high} human basal PESCs. These data suggest that phenotypically and functionally different cell populations might be contained in the purified basal PESC population. The heterogeneously expressed proteins might be novel markers, identified by such subpopulations. We found that CD13 was heterogeneously expressed in human basal PESCs (Fig. 1A). Additionally, human CD49f$^+$/TROP2^{high} basal PESCs show differential Syndecan-1 (CD138) expression. While 15–50% of all human basal PESCs were negative for Syndecan-1, the Syndecan-1 positive fraction was mainly found in the TROP2^{high} cells. CD138 expression levels are positively correlated with that of TROP2 (Fig. 1B).

Specific heterogeneous expression of stage-specific embryonal antigens on murine and human basal PESCs

Stage-specific embryonal antigens (SSEAs) are expressed as carbohydrate adhesion molecules on glycoproteins, glycolipids and proteoglycans of the cell membrane. Stage-specific embryonal antigens are known and established as markers for murine as well as human embryonic stem (ES) cells [14–16]. Given the observed similarities of gene expression profiles of murine as well as human basal PESCs with the profiles of ES cells [11], we were interested in the specific expression of SSEAs we discovered in the cell surface protein screen. Murine basal PESCs demonstrate a heterogeneous SSEA-1 expression (Fig. 1C), whereas human basal PESCs do not express SSEA-1 (Table 2). Overall, 80% of Sca-1$^+$/CD49f$^+$/Trop2^{high} cells are positive for SSEA-1. In contrast, SSEA-4 was found to be more heterogeneously expressed in human CD49f$^+$/TROP2^{high} basal PESCs (Fig. 1D).

High SSEA-4 expression marks a distinct population of human CD49f$^+$/TROP2^{high} basal PESCs with higher *in vivo* regenerative capacity

Antigens of the SSEA family have been described to mark undifferentiated ES cells [17]. We thus investigated if SSEA expression would also be of functional significance in basal PESCs. We FACS sorted separate populations of LeGO-V2 transduced murine Sca-1$^+$/CD49f$^+$/Trop2^{high}/SSEA-1^{low} and Sca-1/CD49f$^+$/Trop2^{high}/SSEA-1^{high} basal PESCs (Fig. 2A). Comparison of the SSEA-1^{low} and SSEA-1^{high} populations in their *in vivo* regenerative potential using s.c. transplantations in nude mice with comparable cell numbers revealed no significant difference (data not shown). Additionally, we FACS sorted populations of LeGO-V2 transduced human CD49f$^+$/TROP2^{high}/SSEA-4^{low} basal PESCs and compared their potential to regenerate prostate gland structures with the sorted CD49f$^+$/TROP2^{high}/SSEA-4^{high} population *in vivo* (Fig. 2B). In contrast to the murine SSEA-1, expression of human SSEA-4 demonstrates a correlation with the FACS forward scatter (size of cells). The limiting dilution transplantation experiments revealed an increased regenerative capacity of CD49f$^+$/TROP2^{high}/SSEA-4^{high} cells to form androgen receptor positive prostatic tubules *in vivo* as compared with the pooled CD49f$^+$/TROP2^{high} as well as CD49f$^+$/TROP2^{high}/SSEA-4^{low} basal PESC populations (Table 4, Fig. 2C and D).

Discussion

Based on a new method to enrich and expand primary basal prostate progenitor cells from murine and human origin, we investigated their surface protein profile. This profile may serve as a reference for future

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

Table 1 Cell surface protein profile of murine basal PESCs

protein		protein		protein		protein		protein	
CD2		CD51		CD140		I-A/I-E		CD55	
CD4		CD53		CD144		IL-21R		CD61	
CD5		CD62E		CD147		Intß7		CD69	
CD8a		CD62L		CD153		LPAM		CD79b	
CD9		CD70		CD162		Sca-1		CD81	
CD11a		CD71		CD172		Ly-6D		CD95	
CD11b		CD72b		CD179		Ly-6G		CD119	
CD13		CD73		CD180		Ly-6C		CD120a	
CD14		CD80		CD184		CD107		CD120b	
CD16/32		CD83		CD195		MAd		CD152	
CD18		CD86		CD197		MD-1		CD154	
CD19		CD90.2		CD200		NKG2		CD279	
CD21/35		CD94		CD209		NK-T		CD22.2	
CD23		CD98		CD210		PIR-A		CD45.1	
CD24		CD102		CD223		PreBCR		CD45.2	
CD25		CD103		CD244		Siglec-F		CD64a/b	
CD26		CD104		CD252		Syndecan-4		CD72a/b/d	
CD29		CD106		CD253		T/B cell act.		CD157	
CD31		CD117		CD254		Ter119		CD212	
CD34		CD121		CD267		IgD		CD244.2	
CD35		CD122		CD273		IgE		H-2D^{b}	
CD38		CD123		CD274		IgM		H-2K^{b}	
CD41		CD124		CD278		CD28		H-2K^{d}	
CD43		CD125		CD284		KLRG1		H-2K^{k}	
CD44		CD126		CD309		CD3e		H-2K^{Q}	
CD45		CD127		CD314		CD11c		H-2K^{S}	
CD45R		CD131		CD326		CD27		IFN-a/ß	
CD45RA		CD132		CD335		CD30		Ly-49c	
CD45RC		CD134		4-188		CD40		Ly-51	
CD47		CD135		P65		CD42d		NK-1.1	
CD49b		CD137		F4/80r		CD48		SSEA-1	
CD49d		CD138		GITR		CD54		SSEA-4	
Pre-TCR		QA-1B							

FACS Array results of all three plates of the murine BD LyoplateTM Cell Surface Marker Screening Panel. Uncoloured spots/proteins did not demonstrate cell surface protein positivity as compared with the corresponding isotype control = negative. Red coloured spots/wells demonstrate a positive expression of surface proteins on murine PESCs as compared with the isotype control, $P < 0.001$. Saturation of the red colour demonstrates the strength of expression as measured by FACS. Blue coloured spots/wells show cell surface proteins with an heterogeneous expression on murine PESCs including low and high expressing cells.

investigations on basal PESCs. We previously demonstrated that *in vitro* expanded basal PESCs retain stem cell activity [11]. In addition to the known proteins CD49f and TROP2, we here describe a number of additional antigens (Tables 1 and 2) that are expressed on those cells. This extended surface marker panel might be of use for future studies, especially those using flow cytometry methodologies. Basal PESCs are suggested to be cells of origin of prostatic diseases by being substantially involved in the development of prostate cancer as well as glandular benign prostatic hyperplasia [1–3, 18]. Therefore, the identification of the cell surface marker profile of basal PESCs is helpful for future scientific studies by providing additional markers for identification and purification. For example, both laminin binding integrins, CD49c and CD29, are observed both in mouse and human PESCs. Interestingly, we also find heterogeneous expression of the tetraspanin CD151, which is known to bind to integrins including CD49c and modulate their function. Thus, our data suggest a potential link worth investigating in the future. Among the investigated differentially regulated proteins CD13, CD138 and SSEAs only the glycosphingolipid SSEA-4 proved to have a functional influence on the regenerative capacity of basal PESCs to form prostatic glands *in vivo*. Glycosphingolipids are a group of lipids that are involved in the formation of cell membranes. They consist of a hydrophobic ceramide portion and a glycosidically linked carbohydrate. This carbohydrate is presented on the outside of the cell membrane, where it is involved in adhesion or signalling between cells [19, 20]. The addition of a third sugar molecule to the lactose disaccharide of glycosphingolipids determines the further division into more complex sphingolipids, including the SSEA-1 and SSEA-4 investigated in our study. Since the establishment of the first murine ES cell line in 1981, and the demonstration that this cell line specifically expresses glycosphingolipids,

Table 2 Cell surface protein profile of human basal PESCs

protein		protein		protein		protein		protein	
CD1a		CD46		CD107a		CD196		TRA-1-81	
CD1b		CD47		CD107b		CD197		Vß 23	
CD1d		CD48		CD108		CD200		Vß 8	
CD2		CD49a		CD109		CD205		CD49f	
CD3		CD49b		CD112		CD206		CD104	
CD4		CD49c		CD114		CD209		CD120b	
CD4v4		CD49d		CD116		CD220		CD132	
CD5		CD49e		CD117		CD221		CD201	
CD6		CD54		CD118		CD226		CD210	
CD7		CD55		CD119		CD227		CD212	
CD8a		CD56		CD120a		CD229		CD267	
CD8b		CD57		CD121a		CD231		CD294	
CD9		CD58		CD121b		CD235a		CD326	
CD10		CD59		CD122		CD243		Integrinß7	
CD11a		CD61		CD123		CD244		SSEA-3	
CD11b		CD62E		CD124		CD255		CD50	
CD11c		CD62L		CD126		CD268		CD51/61	
CD13		CD62P		CD127		CD271		CD53	
CD14		CD63		CD128b		CD273			
CD15		CD64		CD130		CD274			
CD15s		CD66		CD134		CD275			
CD16		CD66b		CD135		CD278			
CD18		CD66f		CD137		CD279			
CD19		CD69		CD137Lig		CD282			
CD20		CD70		CD138		CD305			
CD21		CD71		CD140a		CD309			
CD22		CD72		CD140b		CD314			
CD23		CD73		CD141		CD321			
CD24		CD74		CD142		CDw327			
CD25		CD75		CD144		CDw328			
CD26		CD77		CD146		CDw329			
CD27		CD79b		CD147		CD335			
CD28		CD80		CD150		CD336			
CD29		CD81		CD151		CD337			
CD30		CD83		CD152		CD338			
CD31		CD84		CD153		CD340			
CD32		CD85		CD154		aßTCR			
CD33		CD86		CD158a		ß2mglobul			
CD34		CD87		CD158b		BLTR-1			
CD35		CD88		CD161		CLIP			
CD36		CD89		CD162		CMRF-44			
CD37		CD90		CD163		CMRF-56			
CD38		CD91		CD164		EGF-R			
CD39		CDw93		CD165		fMLP R			
CD40		CD94		CD166		ydTCR			
CD41a		CD95		CD171		HLA- A,B,C			
CD41b		CD97		CD172b		HLA-A2			
CD42a		CD98		CD177		HLA-DQ			
CD42b		CD99		CD178		HLA-DR			
CD43		CD99R		CD180		HLA-DR, DP			
CD44		CD100		CD181		MIC A/B			
CD45		CD102		CD183		NKB1			
CD45RA		CD103		CD184		SSEA-1			
CD45RB		CD105		CD193		SSEA-4			
CD45RO		CD106		CD195		TRA-1-60			

FACS Array results of all three plates of the human BD Lyoplate™ Cell Surface Marker Screening Panel. Uncoloured spots/proteins did not demonstrate cell surface protein positivity as compared with the corresponding isotype control = negative. Red coloured spots/wells demonstrate a positive expression of surface proteins on murine PESCs as compared with the isotype control, $P < 0.001$. Saturation of the red colour demonstrates the strength of expression as measured by FACS. Blue coloured spots/wells show cell surface proteins with an heterogeneous expression on human PESCs including low and high expressing cells.

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

Table 3 Cross-species comparison of surface proteins expressed by basal PESCs

Protein	Expressed in mice	Expressed in humans	Expressed in both species
CD9			■
CD13		■	
CD24			■
CD26		■	
CD29			■
CD44			■
CD46		■	
CD47			■
CD49b			■
CD49c		■	
CD49d		■	
CD49e		■	
CD49f			■
CD51	■		
CD53	■		
CD54		■	
CD55		■	
CD58		■	
CD59		■	
CD63		■	
CD66		■	
CD71			■
CD73			■
CD74		■	
CD81			■
CD86		■	
CD91		■	
CD95			■
CD98			■
CD99		■	
CD104			■
CD109		■	

Table 3. Continued

Protein	Expressed in mice	Expressed in humans	Expressed in both species
CD119		■	
CD124	■		
CD125	■		
CD126	■		
CD138			■
CD146		■	
CD147			■
CD151		■	
CD153	■		
CD164		■	
CD166		■	
CD171		■	
CD200	■		
CD210	■		
CD221		■	
CD223	■		
CD227		■	
CD271		■	
CD273		■	
CD274		■	
CD321		■	
CD326			■
CD340		■	
β2-microglobulin		■	
Sca-1	■		
H-2K^{b}	■		
H-2K^{d}	■		
HLA- A,B,C		■	

Proteins positively expressed in flow cytometry (FACS Array) as compared with corresponding control, $P < 0.001$.

glycosphingolipids in particular the SSEAs were established in numerous studies as a marker of pluripotent and multipotent stem cells [17]. It has been demonstrated that murine ES cells express SSEA-1 [21]. Interestingly, SSEA-1 is also a marker of the differentiation of

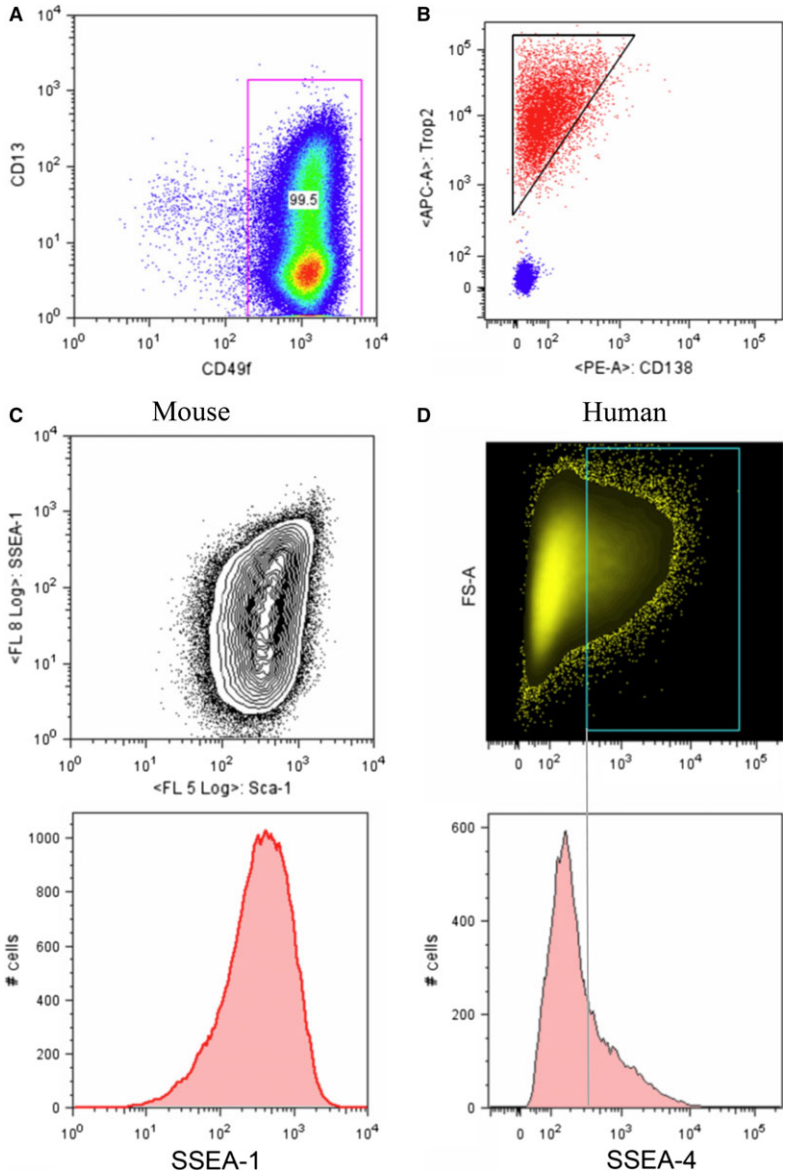

Fig. 1 Heterogeneous expression of cell surface proteins on CD49f^{+}/TROP2^{high} basal PESCs. (**A**) FACS plot demonstrating the heterogeneous expression of CD13 within CD49f^{high} expressing human basal PESCs, CD13-APC=clone WN15; CD49f-PE=clone GoH3, PI^{-} negative gate, $P < 0.001$ as compared with Mouse IgG1 and Rat IgG2a isotype controls. (**B**) FACS plot demonstrating the heterogeneous expression of CD138 (Syndecan-1, red colour as compared with isotype control=blue) and the correlation of higher CD138 expression with TROP2 expression in human basal PESCs. CD138-PE=clone Wi15; TROP2-APC=clone FAB650A (R&D), PI^{-} negative gate, $P < 0.001$ as compared with Mouse IgG1 and Mouse IgG2a isotype controls. (**C**) FACS plots demonstrating the heterogeneous expression of SSEA-1 on murine basal PESCs (below) and the heterogeneous expression of SSEA-1 in correlation to the Sca-1^{high} expression of murine basal PESCs. SSEA-1-APC=clone MC480; Sca-1-PECy7 = clone E13-161.7, PI^{-} negative gate, $P < 0.001$ as compared with Mouse IgM and Rat IgG2a isotype controls. (**D**) FACS plots demonstrating the heterogeneous expression of SSEA-4 on human basal PESCs. Around 20% of all human basal PESCs express SSEA-4. SSEA-4-APC=clone MC813-70; PI^{-} negative gate, $P < 0.001$ as compared with Mouse IgG3 isotype control.

murine ES cells. It was demonstrated that approximately 50% of undifferentiated murine ES cells express SSEA-1; however, less than 10% of the more differentiated cells express SSEA-1 [22]. ES cells of monkeys and humans in contrast express SSEA-3 and SSEA-4 as markers, but not SSEA-1 [16, 23]. Similar to SSEA-1 in the mouse, human SSEA-3 and SSEA-4 expression correlated with the level of

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

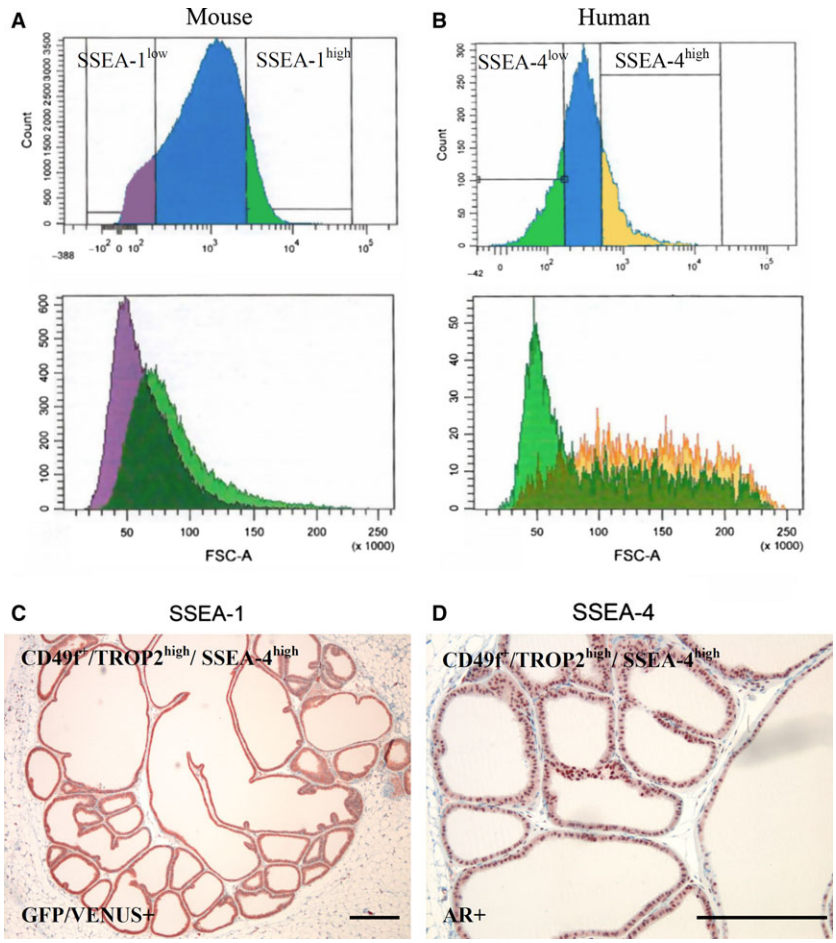

Fig. 2 Sorting and *in vivo* transplantation of different SSEA-expressing basal PESC populations. (**A**) FACS gate of Lin$^-$/PI$^-$/Sca-1$^+$/CD49f$^+$/Trop2^{high} murine LeGO-V2-basal PESCs sorting for SSEA-1^{low} and SSEA-1^{high}. (**B**) FACS gate of Lin$^-$/PI$^-$/CD49f$^+$/TROP2^{high} human LeGO-V2-basal PESCs sorting for SSEA-4^{low} and SSEA-4^{high}. (**C**) GFP/Venus positivity of *in vivo* regenerated prostate ducts derived from 250 transplanted human basal PESCs with the sorted Lin$^-$/PI$^-$/CD49f$^+$/TROP2^{high}/SSEA-4^{high} phenotype, scale bar=500 μm. (**D**) Androgen receptor (AR) positivity of *in vivo* regenerated prostate ducts derived from 250 transplanted human basal PESCs with the sorted Lin$^-$/PI$^-$/CD49f$^+$/TROP2^{high}/SSEA-4^{high} phenotype, scale bar = 500 μm.

differentiation of ES cell lines [24]. These species-specific expressions of SSEAs were confirmed in induced pluripotent stem (IPS) cells. After reprogramming mouse fibroblasts into functional IPS cells, these cells demonstrate positive SSEA-1 expression [25]. In contrast, human IPS cells expressed SSEA-3 and SSEA-4, but showed no SSEA-1 expression (Takahashi *et al.*, 2007; Yu *et al.*, 2007). Stage-specific embryonal antigens were also used as markers in adult stem cells as well as for cancer (medulloblastoma) [26]. Doetsch *et al.* detected SSEA-1 in murine adult neural stem cells [27]. In addition, SSEA-4 expression was detected in human adult mesenchymal stem cells within the bone marrow [28].

Our results demonstrate that up to 80% of expanded murine Sca-1$^+$/CD49f$^+$/Trop2^{high} basal PESCs express SSEA-1. On the other hand, 20–30% of expanded CD49f$^+$/TROP2^{high} human basal PESCs express SSEA-4. We thus for the first time describe SSEA expression on adult PESCs. However, sorting and transplanting different populations of SSEA-1^{low} and SSEA-1^{high} expressing murine basal PESCs did not result in significant changes in *in vivo* regenerative capacity. In addition, SSEA-1 was not specific for epithelial cells in the murine prostate, as also 15% of all lineage negative cells expressed SSEA-1 (data not shown). It is possible that these cells correspond to mesenchymal progenitor cells in the prostate [29], which has to be investigated in future studies. Conversely, we can demonstrate results of a potential superior regenerative stem cell capacity for the human CD49f$^+$/TROP2^{high}/SSEA-4^{high} basal PESCs lineage (0.2–0.4% of all prostatic cells) as compared to the known CD49f$^+$/TROP2^{high} basal PESCs population (1–3% of all prostatic cells) to form prostate tubules *in vivo*. This result, however, remains provisional as the true stem cell frequency of SSEA-4^{high} basal PESCs remains undetermined in our study. Nevertheless, we suggest that SSEA-4 positivity could further narrow down the lineage of basal PESCs towards the true prostate basal epithelial

Table 4 *In vivo* regenerative capacity of FACS sorted (lin^-/PI^-) human prostate epithelial progenitor cell populations

$CD49f^+/TROP2^{high}$		$CD49f^+/TROP2^{high}/SSEA\text{-}4^{low}$		$CD49f^+/TROP2^{high}/SSEA\text{-}4^{high}$	
Cells transplanted	Microscopic GFP+ ducts	Cells transplanted	Microscopic GFP+ ducts	Cells transplanted	Microscopic GFP+ ducts
250	+	250	–	250	+
250	–	250	–	250	+
250	–	250	–	250	+
2500	+	2500	–	2500	+
2500	+	2500	–	2500	+
2500	–	2500	–	2500	–
25,000	+	25,000	–	25,000	+
25,000	–	25,000	–	25,000	–
25,000	–	25,000	–	25,000	–

Sorted populations were transplanted s.c. in nude mice together with E16 UGSM as described [11], $P = 0.01$.

stem cell. Our new methods to expand functional basal PESCs may open up new possibilities for studying the aetiology of prostatic diseases. Discovering the cell surface protein profile of murine and human basal PESCs reveals differentially expressed proteins that may change the biology and regenerative function of these cells within the prostate. Stage-specific embryonal antigen-4 is a candidate cell surface marker that putatively enables a more accurate identification of the basal PESC lineage.

Acknowledgements

We thank Dr. Andreas Trumpp for continued discussions and comments on this article. This work was supported by the SFB 873 'Maintenance and Differentiation of Stem Cells in Development and Disease' funded by the Deutsche Forschungsgemeinschaft (DFG) and the Dietmar-Hopp Foundation.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

Author contribution

T.H. developed the methods and designed the study, performed culture and differentiation experiments *in vitro* and *in vivo* and wrote the article. C.E. helped with lentiviral constructs and performed bioinformatics analyses. C.K. performed experiments and supervised all mouse transplantation experiments. T.R.-W. helped with experiments. A.H. analysed the data and/or provided intellectual guidance regarding their interpretation. M.R.S. designed the study, analysed and evaluated results, and wrote the article.

References

1. **Choi N, Zhang B, Zhang L, *et al.*** Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. *Cancer Cell*. 2012; 21: 253–65.
2. **Goldstein AS, Huang J, Guo C, *et al.*** Identification of a cell of origin for human prostate cancer. *Science*. 2010; 329: 568–71.
3. **Visvader JE.** Cells of origin in cancer. *Nature*. 2011; 469: 314–22.
4. **Wang X, Kruithof-de Julio M, Economides KD, *et al.*** A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature*. 2009; 461: 495–500.
5. **Goldstein AS, Drake JM, Burnes DL, *et al.*** Purification and direct transformation of epithelial progenitor cells from primary human prostate. *Nat Protoc*. 2011; 6: 656–67.
6. **Lukacs RU, Goldstein AS, Lawson DA, *et al.*** Isolation, cultivation and characterization of adult murine prostate stem cells. *Nat Protoc*. 2010; 5: 702–13.
7. **Morrison SJ, Spradling AC.** Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell*. 2008; 132: 598–611.
8. **Chaproniere DM, McKeehan WL.** Serial culture of single adult human prostatic epithelial cells in serum-free medium containing low calcium and a new growth factor from bovine brain. *Cancer Res*. 1986; 46: 819–24.
9. **Litvinov IV, Vander Griend DJ, Xu Y, *et al.*** Low-calcium serum-free defined medium selects for growth of normal prostatic epithelial stem cells. *Cancer Res*. 2006; 66: 8598–607.
10. **Rhim JS, Li H, Furusato B.** Novel human prostate epithelial cell culture models for the study of carcinogenesis and of normal stem cells and cancer stem cells. *Adv Exp Med Biol*. 2011; 720: 71–80.
11. **Hofner T, Eisen C, Klein C, *et al.*** Defined conditions for the isolation and expansion of basal prostate progenitor cells of mouse and human origin. *Stem cell reports*. 2015; 4: 503–18.

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

12. **Weber K, Thomaschewski M, Warlich M, *et al.*** RGB marking facilitates multicolor clonal cell tracking. *Nat Med*. 2011; 17: 504–9.
13. **Kutner RH, Zhang XY, Reiser J.** Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors. *Nat Protoc*. 2009; 4: 495–505.
14. **Henderson JK, Draper JS, Baillie HS, *et al.*** Preimplantation human embryos and embryonic stem cells show comparable expression of stage-specific embryonic antigens. *Stem Cells*. 2002; 20: 329–37.
15. **Shamblott MJ, Axelman J, Wang S, *et al.*** Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci USA*. 1998; 95: 13726–31.
16. **Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al.*** Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282: 1145–7.
17. **Evans MJ, Kaufman MH.** Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981; 292: 154–6.
18. **Isaacs JT.** Prostate stem cells and benign prostatic hyperplasia. *Prostate*. 2008; 68: 1025–34.
19. **Simons K, Toomre D.** Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol*. 2000; 1: 31–9.
20. **Yanagisawa M.** Stem cell glycolipids. *Neurochem Res*. 2011; 36: 1623–35.
21. **Martin GR.** Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA*. 1981; 78: 7634–8.
22. **Cui L, Johkura K, Yue F, *et al.*** Spatial distribution and initial changes of SSEA-1 and other cell adhesion-related molecules on mouse embryonic stem cells before and during differentiation. *J Histochem Cytochem*. 2004; 52: 1447–57.
23. **Thomson JA, Kalishman J, Golos TG, *et al.*** Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci USA*. 1995; 92: 7844–8.
24. **Draper JS, Pigott C, Thomson JA, *et al.*** Surface antigens of human embryonic stem cells: changes upon differentiation in culture. *J Anat*. 2002; 200: 249–58.
25. **Takahashi K, Yamanaka S.** Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126: 663–76.
26. **Read TA, Fogarty MP, Markant SL, *et al.*** Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. *Cancer Cell*. 2009; 15: 135–47.
27. **Doetsch F, Caille I, Lim DA, *et al.*** Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 1999; 97: 703–16.
28. **Gang EJ, Bosnakovski D, Figueiredo CA, *et al.*** SSEA-4 identifies mesenchymal stem cells from bone marrow. *Blood*. 2007; 109: 1743–51.
29. **Anjos-Afonso F, Bonnet D.** Non-hematopoietic/endothelial SSEA-1+ cells define the most primitive progenitors in the adult murine bone marrow mesenchymal compartment. *Blood*. 2007; 109: 1298–306.