Identification and isolation of human prostate epithelial stem cells based on $\alpha_2\beta_1$-integrin expression

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SUMMARY

A major impediment to our understanding of the biology of stem cells is the inability to distinguish them from their differentiating progeny. We made use of the known association of stem cells with basement membranes to isolate prostate epithelial stem cells. We show that, in vivo, putative stem cells express higher levels of the α_2-integrin subunit than other cells within the basal layer. Approximately 1% of basal cells examined by confocal microscopy were integrin 'bright', and these cells can be selected directly from the tissue on the basis of rapid adhesion to type I collagen. This selected population has a basal phenotype, as determined by expression of CK5 and CK14 and lack of expression of the differentiation-specific markers prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), and has a fourfold greater ability to form colonies in vitro than the total basal population. These putative stem cells are distinguished from other basal cells by their ability to generate prostate-like glands in vivo with morphologic and immuno-histochemical evidence of prostate-specific differentiation. These properties are consistent with a stem cell origin. Furthermore, the presence of surface integrins on prostate stem cells suggests that these cells share common pathways with stem cells in other tissues.

Key words: Stem cells, Integrins, Prostate

INTRODUCTION

Classically, stem cells have been studied in tissues that undergo rapid cell turnover, such as bone marrow, skin and the gastrointestinal tract (Dexter et al., 1977; Hall and Watt, 1989; Potten and Loeffler, 1990). More recently, it has been recognised that stem cells are also present in tissues that normally undergo very limited regeneration or turnover, such as the prostate (Isaacs, 1987). This is best illustrated by animal studies of the effect of androgen on the prostate. Castration of male rats leads to a rapid involution of the gland with only the basal epithelial cells surviving. Upon androgen replacement the gland is restored to its normal function (English et al., 1989; Walensky et al., 1993). Only the differentiated secretory luminal cells are affected by androgen removal, which suggests that regeneration of the gland must result from proliferation and differentiation of the surviving basal cells (Bonkoff and Remberger, 1996).

In self-renewing epithelial tissues, such as the gut and epidermis, the small stem cell population has the capacity for extended or unlimited growth and its progeny are either stem cells or cells with more limited proliferative capacity, termed transit amplifying cells. These daughter cells divide to maintain tissue balance but are limited to a finite number of cell divisions before they terminally differentiate (Potten, 1981; Hall and Watt, 1989). Cell types are organised whereby stem cells, transit amplifying cells and mature terminally differentiated cells occupy discrete locations within the tissue, often forming stratified layers (Potten and Morris, 1988). A similar hierarchical arrangement has been postulated for the more slowly dividing adult prostate (Isaacs, 1987; Bonkoff and Remberger, 1996). However, we have only recently begun to accumulate evidence in support of a transit amplifying compartment in the prostate (Verhagen et al., 1988; Robinson et al., 1998; van Leenders et al., 2000).

The characterisation of tissue stem cell populations remains difficult because of the lack of markers that can distinguish between stem cells and their differentiating progeny. For many tissues, panels of molecular markers have been developed to define the stem cell compartment. For example, integrins, which mediate the attachment of cells to extracellular matrix (ECM) proteins on the basement membrane, have been instrumental in the identification of stem cells in skin (Jones et al., 1995; Li et al., 1998) and testis (Shinohara et al., 1999). Epidermal stem cells express higher levels of integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ than transit-amplifying cells and this can be used to determine the location of stem cells within the epidermis, and to isolate them directly from the tissue on the basis of rapid adherence to type IV collagen (Jones et al., 1995).

As stem cells of different tissues show certain similarities in biological behaviour, we hypothesised that they might share similar molecular properties. For instance, stem cells are usually on the basement membrane situated in a protected region, or niche, among supporting cells. In the present study

we investigated whether there was a correlation between the adhesiveness of prostate cells to ECM proteins and their ability to form prostate-like glands in vivo.

The major integrins in human prostate are $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_6\beta_4$ (Bonkhoff et al., 1993; Knox et al., 1994). We focused on integrin $\alpha_2\beta_1$, which mediates adhesion to type IV collagen, type I collagen and laminin 1 and is restricted to the basal cells of the prostate (Knox et al., 1994).

MATERIALS AND METHODS

Tissue collection, isolation and culture of epithelial cells

Human prostate tissue was obtained from 30 patients (age range 59-86), undergoing transurethral and retropubic prostatectomy for benign prostatic hyperplasia (BPH) and cystoprostatectomy for bladder cancer. The condition of BPH was confirmed by histological examination of representative fragments. Patients who had undergone any hormonal therapy were excluded. Collagenase digestion of prostatic tissue released epithelial structures (organoids; acini and ducts) which were separated from the stromal fraction by repeated unit gravity centrifugation. High purity (>98%) is obtained by this method (Collins et al., 1994). Organoids were then dis-aggregated into a single cell suspension by incubation with trypsin/EDTA (Life Technologies, Paisley, UK) for 30 minutes at 37°C.

Immunomagnetic positive selection of basal cells

MACS microbeads (Miltenyi Biotec Ltd, Surrey, UK), linked to anti-CD44 (Liu et al., 1997) were used to isolate basal cells from luminal cells. Epithelial cells were labelled with anti-human CD44 (clone G44-26; Pharmingen, Becton Dickinson, Oxford, UK), followed by incubation with MACS goat anti-mouse IgG microbeads. The cell suspension was then applied to a MACS column. Labelled basal cells were eluted and re-suspended in WAJC 404 complete medium (Robinson et al., 1998).

Cell adhesion to ECM proteins

CD44 positive basal cells were plated onto dishes coated with type I collagen (52 μg/ml), type IV collagen (88 μg/ml) and laminin 1 (100 μg/ml; Biocoat®, Becton Dickinson) which had previously been blocked with 0.3% bovine serum albumin (fraction V, Sigma-Aldrich, Poole, UK) in Dulbecco's phosphate buffered saline (PBS; Oxoid Ltd, Basingstoke, UK). We had determined previously that 50-100μg/ml of each protein was sufficient for maximum attachment (results not shown). After 5 minutes, dishes were washed with PBS and adherent cells were either harvested with trypsin-EDTA for grafting, or fixed in methanol at –20°C. Non-adherent cells, recovered during washing, were plated onto ECM-coated dishes and were either used for grafting or fixed in methanol at –20°C.

Basal cells were also counted and plated for the determination of colony forming efficiency (CFE). After plating, single cells were marked and examined at intervals of up to 21 days when they were subsequently fixed and colonies scored under a phase-contrast microscope. Colonies were scored if they contained >32 cells. As the number of cells selected was small, irradiated (60 Grays) STO cells were added as feeders. In control dishes the unattached cells were not removed by washing.

To determine the optimum time to select the stem cell population, basal cells were plated onto ECM-coated dishes at 37°C for 5, 20, 60 and 3 hours and the percentage of adherent, CK18-expressing cells was determined.

Xenografts

To determine the ability of rapidly-adherent basal cells to induce ductal branching morphogenesis, growth and functional cytodifferentiation in vivo, epithelial cells (1×10^5 to 1×10^6), obtained from adult prostate after immunomagnetic selection of the basal cell fraction, were directly plated onto petri dishes coated with type I collagen. Nonadherent cells were removed after 5 minutes. The adherent cells were trypsinised and combined with cultured stromal cells (passage 1-4), at a ratio of 1:1 epithelium to stroma and injected subcutaneously into 6-8 weeks old male, nude mice (strain ICRF-nu). The mice were sacrificed 6 weeks after grafting. Grafts were removed, fixed in phosphate-buffered formalin, embedded in paraffin, and sectioned. Serial sections (4 μm) were stained and examined for the development of organised prostatic glandular tissue. The capacity to differentiate in vivo to the secretory phenotype (expression of androgen receptor, prostate specific antigen (PSA) and prostatic acid phosphatase (PAP)) was taken as evidence for an epithelial stem cell population. Cells that had not adhered after 20 minutes to type I collagen were used as controls.

Immunofluorescent staining of tissue sections

Prostate cells expressing the α_2-integrin subunit were identified by direct immunofluorescent staining using anti-α_2 mouse monoclonal antibody (clone AK-7; TCS Biologicals Ltd, Buckingham, UK) directly conjugated with fluorescein isothiocyanate (FITC). Confocal microscopy was used to quantify fluorescence by recording the intensity of pixels along a transect through lateral cell borders. A series of 1 μm optical sections through the entire thickness of the tissue was obtained using the 60× objective of the confocal microscope, and a Z series was constructed from these sections. In order to provide a clear definition of integrin-bright cells, and account for variation in intensity as a consequence of bleaching, bright cells were defined as those in which the fluorescence intensity across cell borders was at least twice the average of other cells in the transect.

Immunocytochemical staining and FACS of isolated cells and grafts

Tissue sections taken from xenografts were fixed in formalin and paraffin-embedded. Isolated cells and colonies were fixed in methanol at –20°C. After incubating with the primary antibody, a biotinylated antibody was applied to the specimens followed by incubation with avidin-biotin complex reagents (Dako Ltd, UK). The staining was developed with diaminobenzidine tetrahydrochloride (DAB; Sigma).

The anti-cytokeratin antibodies used were 34βE12 (which identifies cytokeratins 1, 5, 10 and 14 of the basal cell compartment in prostate; Dako Ltd), CY-90, designated CK18, which reacts with cytokeratin 18 and identifies differentiating epithelium in prostate (Sigma) (Robinson et al., 1998) and RCK 108 (designated CK19) which binds to basal and luminal epithelial cells and recognises cytokeratin 19. Anti PSA (clone ER-PR8) and anti PAP (clone PASE/4LJ) antibodies are markers of secretory luminal cells (Dako Ltd). Anti androgen receptor (clone AR27) antibody was obtained from Novocastra Laboratories Ltd, Newcastle upon Tyne, UK). The mesenchymal markers vimentin and smooth muscle α actin was detected by antibodies V9 and 1A4 respectively (Sigma). Basal cells were analysed on a Becton-Dickinson FACScan. At least 10,000 events were acquired for each sample. Cells positive for propidium iodide were gated out. The antibodies used for FACS analysis were anti-α_2 mouse monoclonal antibody (clone AK-7; TCS Biologicals Ltd), anti-α_3 mouse monoclonal antibody (clone Mikd2; TCS Biologicals Ltd) and anti-α_6 mouse monoclonal antibody (clone 4F10; TCS Biologicals Ltd). The antibodies were directly conjugated with FITC.

RESULTS

α_2-Integrin-bright cells are located within the basal layer of prostate epithelium

We initially quantified the levels of the α_2-integrin subunit, within epithelial cells on the basement membrane of acini

using an antibody to α_2, directly conjugated to FITC. We used a directly conjugated antibody to obviate any amplification provided by a secondary antibody that might mask any small, but potentially relevant differences in integrin levels in the tissue (Jones et al., 1995). α_2-Integrin expression was measured along the lateral cell borders of cells within the basal layer as expression was confined to the basolateral surfaces of the cells (Fig. 1). Only 1.07% of the basal cells examined were defined as α_2-integrin bright. Bright cells were never found together, and between 0-5 were present in each cross section of an acinus. There was no significant difference in the number of bright cells found and the size of acini (P>0.05). Based upon the average circumference of acini examined (626 μm±337 μm), 0.64% (±0.30%,) of the total cells in each acinus were examined in the plane of a cross-section; 43% of the 300 acini examined contained integrin-bright cells. Assuming that bright cells are evenly distributed between acini, using these figures we estimated that each acinus would contain approximately 70 integrin-bright cells.

Clonogenic cells can be enriched by rapid attachment to type I collagen

Previous studies have shown that tissue stem cells can adhere to basement membrane proteins more than other basal cells and that this adhesion might be mediated through differential expression of specific integrins (Jones et al., 1995; Shinohara et al., 1999). To ascertain whether we could use this property to select a stem cell population, we plated basal cells onto culture dishes coated with ECM proteins for different lengths of time. Cells that adhered rapidly to type I collagen, type IV collagen and laminin 1 had a higher CFE than controls (Fig. 2). Within 5 minutes, approximately 3% of the basal population had adhered to type I collagen and the CFE was 3.8-fold greater than the non-selected basal population. Similarly, a 2.5-fold enrichment of colony forming cells was obtained with type IV collagen (5% of the total basal population), whereas the maximum enrichment of colony forming cells with laminin-1 was less than 2-fold and occurred after 5 minutes, when 24% of basal cells had adhered. In contrast to the rapidly adherent population, cells that adhered to ECM after 20 and 60 minutes formed few colonies. Adhesion to type I collagen was used in further experiments because it gave the greatest enrichment for colony-forming cells.

The phenotype of basal cells isolated directly from the prostate was also examined and the results are shown in Fig. 3 and Fig. 4. Epithelial cells that adhered within 5 minutes to type I collagen had a basal phenotype, as determined by expression of CK5 and CK14 (34βE12 antibody) and lack of expression of the differentiation-specific markers PSA and PAP (Fig. 3). However, a small proportion of the rapidly adherent population expressed CK18 (Fig. 4). CK18 is expressed by the differentiated secretory luminal cells in prostate (Wernert et al., 1987), but is also present in a subset of basal cells in situ (Nagle et al., 1991; Yang et al., 1997; van Leenders et al., 2000) and a sub-set of basal cells isolated directly from tissue (Robinson et al., 1998) and is likely therefore to be a marker of basal cells committed to differentiation. It was impossible to retrieve cells before 5 minutes, and in all the experiments undertaken (n=10) approximately 5% of the cells were CK18 positive. Increasing the time of adherence to type I collagen increased the fraction of CK18 cells. Within 20 minutes, 25% of adherent cells expressed CK18. When we compared the expression of CK18 with the unselected population we found that almost 50% of basal cells expressed this differentiation marker (Fig. 4).

Rapidly adherent cells are α_2-integrin bright

To confirm the relationship between integrin levels and proliferative potential, expression of α_2-integrin was analysed by flow cytometry from six tissue samples (Fig. 5A). The results clearly show that the unselected cells have a broad range of α_2 expression, whereas rapid adhesion to type I collagen selected the α_2-integrin-bright cells. From these data we calculated that approximately 15±10% of the total basal cell population was α_2 bright.

It has been reported previously that keratinocytes that

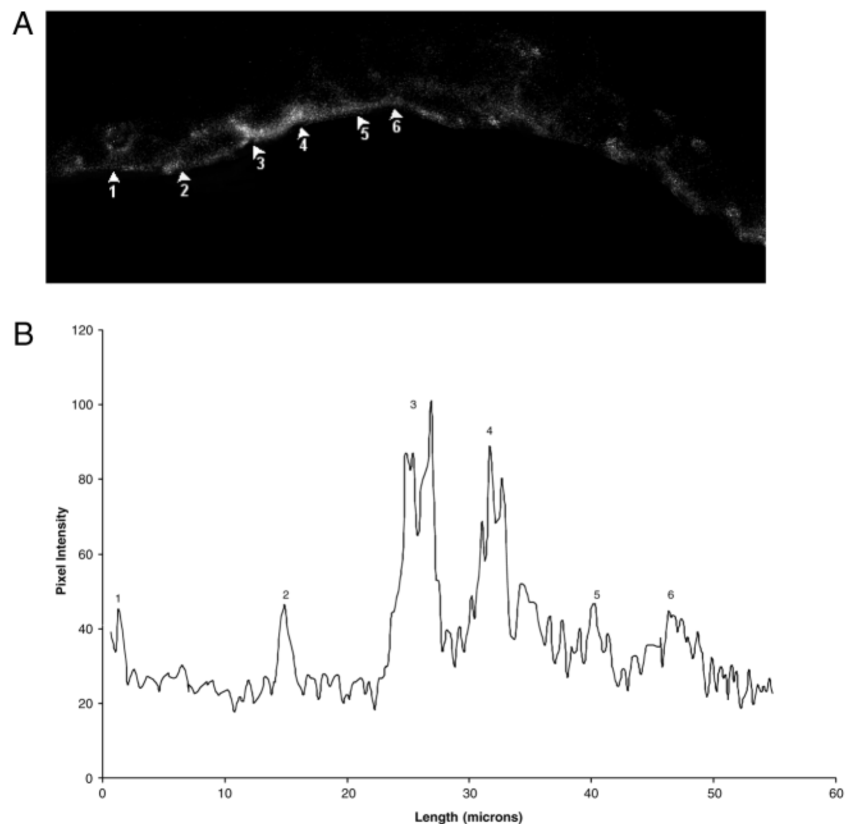

Fig. 1. Frozen sections of prostate were labelled with anti α_2-integrin antibody, directly conjugated to fluorescein isothiocyanate (FITC), and viewed by confocal microscopy. The intensity of fluorescence was measured along a line drawn through the lateral cell borders. (A) Section of human prostate stained with a FITC-conjugated antibody to the α_2-integrin subunit. (B) Fluorescence was measured along basolateral cell borders, with each numbered peak corresponding to one cell border. Peaks 3-4 shows a brightly stained cell surrounded by weakly stained cells (peaks 1-2, 5-6).

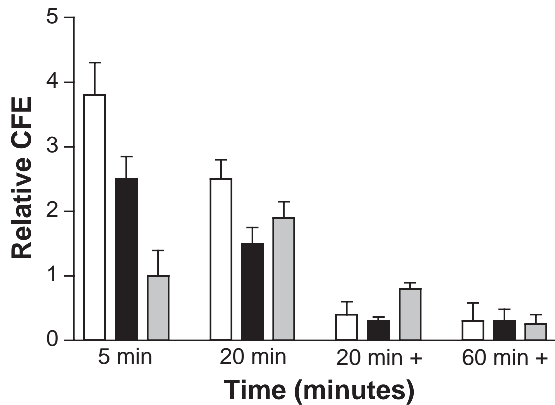

Fig. 2. Basal cells were plated onto type I collagen (open bars), type IV collagen (closed bars) and laminin-1 (grey bars) for 5 and 20 minutes and the nonadherent cells were washed off and plated onto fresh ECM-coated plates (marked as 20 min+ and 60 min+). Cells were counted before the addition of irradiated feeders and were then cultured for up to 21 days. Controls were plated with no pre-selection. Colonies containing 32 or more cells were scored. Colony forming efficiency (CFE) was calculated as the number of colonies formed per number of adherent cells ×100%. CFEs are expressed as the ratio of the control CFE. Results shown are the means±s.e.m. of five experiments.

express high levels of integrins α_3 (Jones et al., 1995) and α_6 (Li et al., 1998) have characteristics of stem cells. We therefore examined their expression pattern by flow cytometry. We wished to determine whether basal cells that rapidly adhere to type I collagen, and are α_2-integrin bright, also express higher levels of integrins α_3 and α_6 than the unselected basal population. Fig. 5B,C illustrates that the unselected basal cells exhibit a broad range of expression for integrins α_3 and α_6 but rapid adhesion to type I collagen selects for cells with higher levels of integrins α_3 and α_6. However, the levels of expression were not as high as for integrin α_2.

Rapidly adherent cells grow more slowly in culture than total basal cells

We compared the types of colony produced by the total basal population and by basal cells selected by rapid adherence to type I collagen (Fig. 6A,B). Colonies were scored if they contained more than 32 cells after 21 days growth. We identified three types of colony. Type I colonies were founded by cells that did not divide in culture for at least 7 days. After this initial lag period they formed large colonies by 28 days (>15 cell doublings). The perimeter of these colonies was nearly circular and many small cells were observed throughout the colony and in some colonies they had concentrated around the perimeter. The interior was often stratified; the upper differentiating layers consisting of larger, flattened cells. By contrast, type II colonies grew more rapidly and were often larger than type I colonies at 21 days. The perimeter was irregular, as the colonies were heterogeneous; small cells were intermingled with differentiating, larger, flattened cells. Type III colonies were small, irregular and terminal. Such colonies contained <32 cells, and all the cells were large, squamous and terminally differentiated after a few rounds of cell division (data not shown). Although we were able to select proliferative basal cells by their rapid attachment to type collagen I, we were unable to select for type I or type II colonies. That is, cells that adhered to type I collagen, within 5 minutes formed either type I or type II colonies. By contrast, cells that attached to type I collagen after 20 minutes founded type III colonies.

Basal cells selected by rapid adhesion to collagen type I form fully differentiated glands in vivo

One of the properties attributed to stem cells is the ability to

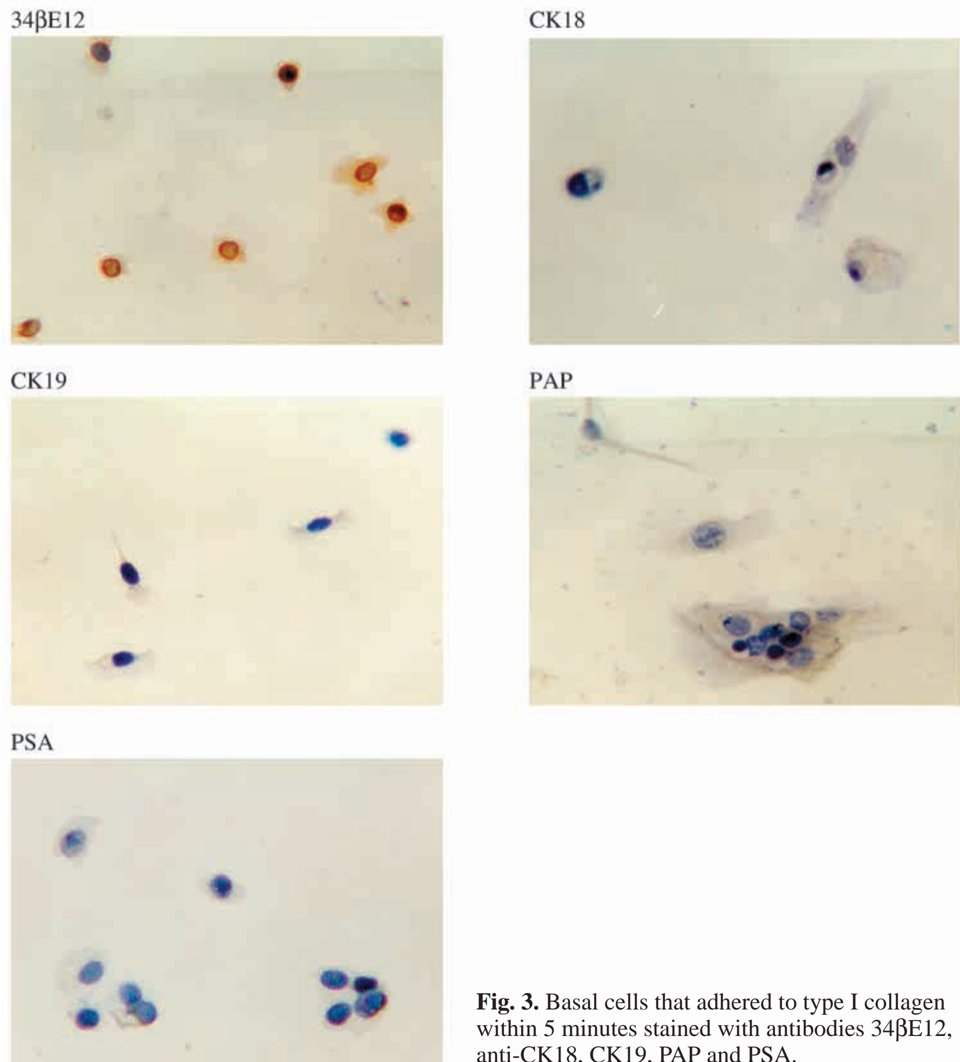

Fig. 3. Basal cells that adhered to type I collagen within 5 minutes stained with antibodies 34βE12, anti-CK18, CK19, PAP and PSA.

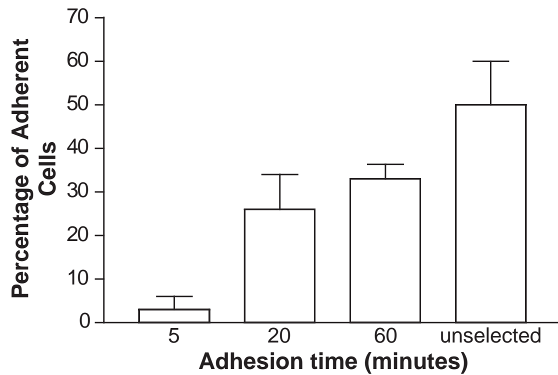

Fig. 4. Percentage of freshly isolated basal cells expressing CK18 (closed bars) after 5, 20, 60 minutes and overnight (marked as unselected) attachment to type I collagen. Results shown are the means±s.e.m. of 10 experiments.

regenerate the different cell types that constitute the tissue in which they exist (Morrison et al., 1997). We therefore determined whether the integrin-bright population, isolated directly from the basal layer, had the potential to self-renew and form fully differentiated glands when grafted into athymic male mice. Adhesion to type I collagen was used in this series of experiments because it gave the greatest enrichment of colony forming cells. Basal cells were selected after 5 minutes on type I collagen-coated plates and were immediately grafted, together with human stromal cells, into the flanks of athymic male mice. After 42 days, a fully formed epithelium was retrieved from 8/30 type I collagen-selected cells. In the remaining cases, no epithelium was recovered, but a dense inflammatory infiltrate was observed. In control samples (basal cells that had not adhered within 20 minutes to type I collagen) no epithelium (0/30) was recovered, but a fibromuscular stromal matrix was apparent in 4/30 of these grafts. In the eight grafts containing epithelium, variable gland formation was observed, with morphologic and immuno-histochemical evidence of both secretory and squamous cell differentiation (Fig. 7). The xenografts often consisted of a single acinus surrounded by minimal, concentrically arranged, loose connective tissue or several acini within connective tissue. Lumina were well defined, often containing a mass of either dead cells or secreted material. The majority were lined with flattened or cuboidal cells, often 3-4 layers thick. These cells stained intensely with the basal cell-specific marker (34βE12 antibody). The cells facing the lumen were morphologically columnar and stained with antibodies against PAP and PSA, which are secreted by the prostate into seminal fluid (Lilja and Abrahamsson, 1988). As the secretory luminal cells are dependent upon androgen for survival we expected that androgen receptor expression would also be expressed in these grafts; indeed this was the case. Expression was confined to the nucleus of some basal cells as well as the secretory luminal layer.

To ascertain the origin of the stromal component, propidium iodide-stained sections were examined from all grafts under investigation. While the epithelial structures always showed the diffusely fluorescent nuclei characteristic of human origin, the stroma showed both the human diffuse stain in addition to the speckled pattern indicative of rodent origin. Stromal cells with the appearance of human nuclei were closely associated with

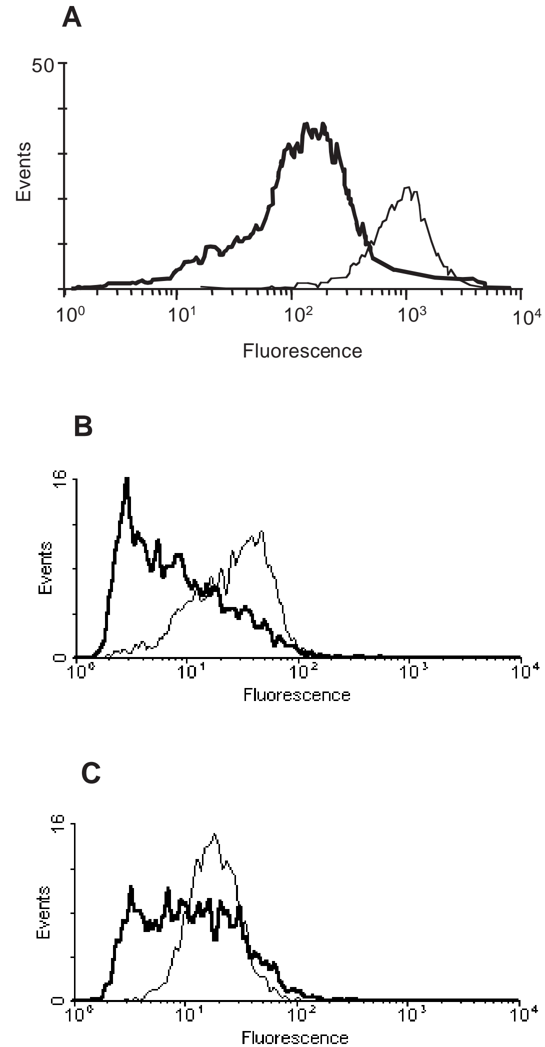

Fig. 5. Basal cells were labelled with FITC-conjugated antibodies to the α_2-, α_3- and α_6-integrin subunits and analysed by flow cytometry. (A) Analysis of α_2-integrin expression. Bold solid line, unselected basal cells; solid line, cells that adhered to type I collagen within 5 minutes. (B) Analysis of α_3-integrin expression. Bold solid line, unselected basal cells; solid line, cells that adhered to type I collagen within 5 minutes. (C) Analysis of α_6-integrin expression. Bold solid line, unselected basal cells; solid line, cells that adhered to type I collagen within 5 minutes.

the epithelial structures. Examination of these cells using antibodies against vimentin and smooth muscle α-actin confirmed that the cells closely associated with the epithelial structures had a smooth muscle phenotype. Vimentin expression, characteristic of stromal fibroblasts was not observed.

DISCUSSION

We have shown that in vivo, high surface expression of the α_2-integrin subunit on human prostate epithelium correlates with colony forming ability and the potential to regenerate a fully differentiated prostate epithelium in vivo. $\alpha_2\beta_1$-integrin-bright cells could be isolated directly from the tissue by rapid

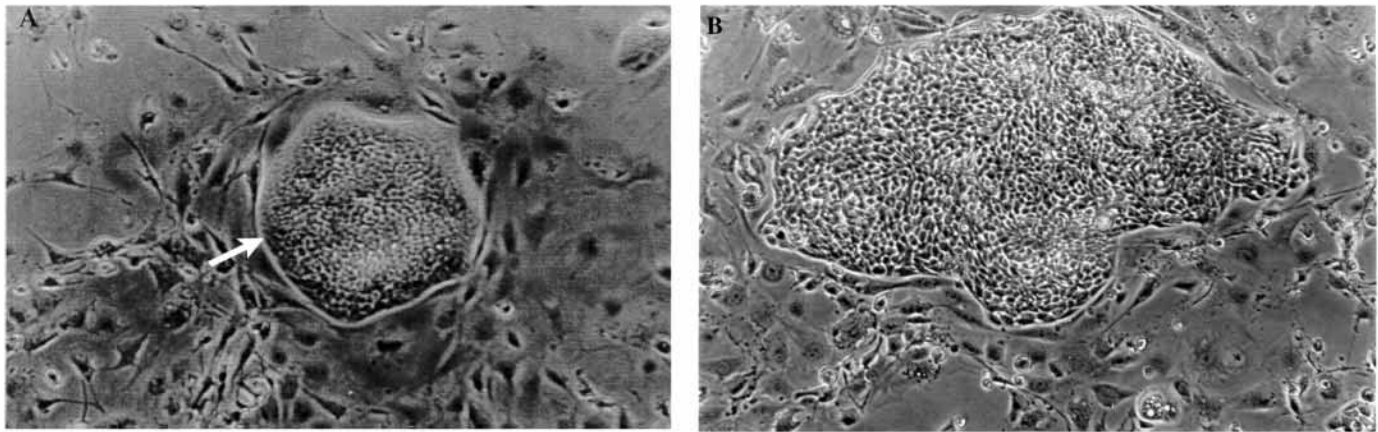

Fig. 6. Colony types founded by basal cells that adhered within 5 minutes to type I collagen. Type I (A) and type II (B) colonies are shown at 21 days growth.

adherence to type I collagen. A fourfold enrichment of colony forming cells was obtained from cells that adhered within 5 minutes to type I collagen, whereas those cells that had not adhered within 20 minutes did not form actively growing colonies. Hudson et al. also demonstrated enrichment of colony forming cells on type I collagen (Hudson et al., 2000). Although there is no single definition for a stem cell, there is general agreement that such a cell would exhibit clonogenicity. However, there is uncertainty in defining the minimum number of generations that defines a cell as a stem cell. Clones containing as few as 32 cells (5 cell doublings) have been interpreted as of stem cell origin (Jones et al., 1995). However, epidermal colonies founded by transit-amplifying cells have been shown to undergo as many as 15 doublings (Barrandon and Green, 1987). Whilst we could show that proliferative cells could be selected by rapid attachment to type I collagen, type I and type II colony-forming cells were not preferentially selected by this method. Although type II colonies were often larger at 21 days, when the colonies were scored they were more heterogeneous than type I colonies; small cells were intermingled with differentiating cells. The other important difference between the colony types was that it took longer for the cells that founded type I colonies to begin a phase growth in vitro, possibly reflecting the slow cell cycle time of stem cells in vivo. Whether type I colonies are founded by stem cells and type II colonies are founded by transit cells can be determined only by studying their long-term proliferative potential rather than their colony forming ability after 21 days growth in vitro. Nonetheless, it is unlikely that all type II colonies were founded by transit

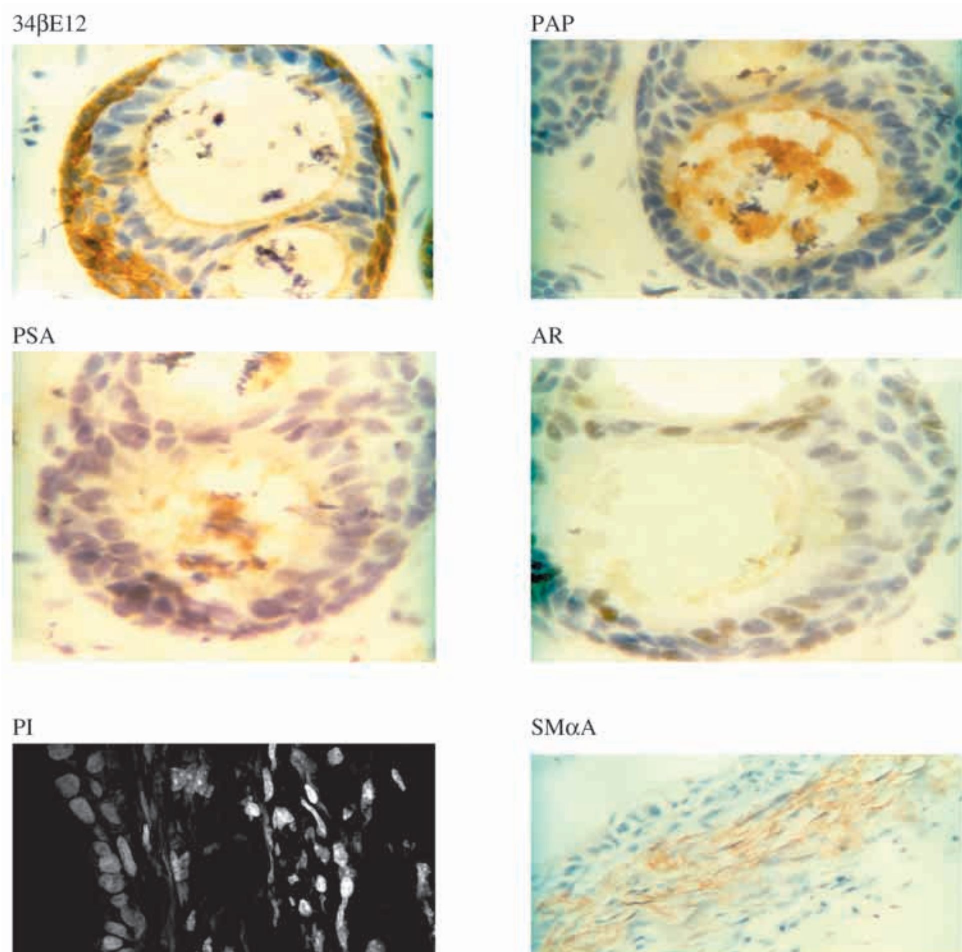

Fig. 7. Xenografts of prostate acini formed by transplantation of basal cells selected by adhesion to type I collagen for 5 minutes and stained with antibodies 34βE12, anti-PAP, anti-PSA, anti-AR, and smooth muscle α actin (SMαA). Grafts were also stained with propidium-iodide (PI). Basal and secretory luminal cells are present.

amplifying cells as the majority of rapidly adherent cells did not express the differentiation marker K18. The cells expressing K18 accounted for less than 5% of the rapidly adherent population. We concluded from these results that, first, the most adhesive cells in our experiments included the stem cells as well as a small population of transit-amplifying cells and, secondly, commitment to differentiation occurs within the basal layer.

One important property attributed to stem cells is the ability to regenerate the different cell types that constitute the tissue in which they exist. Thus, transplanted cells should be capable of self-renewal and to produce progeny that differentiate into a fully functional epithelium. By definition, only stem cells could produce this result. The most adhesive cells in our experiments fulfilled these requirements, as they were able to regenerate a fully differentiated prostate epithelium when grafted into nude mice. By contrast, the slowly adherent cells were incapable of forming prostate epithelium in vivo. These results provide the strongest evidence yet that stem cells are present in the slowly cycling prostate epithelium and that they can be selected by high surface expression of integrin $\alpha_2\beta_1$.

As prostatic development and maintenance of function in the adult is dependent upon stromal interactions (Cunha et al., 1983), epithelial cells were grafted together with stroma to induce epithelial morphogenesis and cytodifferentiation. Although we cannot comment on the nature of this interaction without further study, it was interesting to note that the stromal cells closely associated with the grafts were smooth muscle cells, which begs the question of whether this phenotype is induced by the epithelium (Hayward et al., 1992).

In classical self-renewing tissue, stem cells reside in an optimal microenvironment, or niche. The dividing cells are located in one place and the stem cells lie elsewhere. The histological structure of most epithelia is clearly composed of structural units, and the number of stem cells is directly related to tissue architecture (Slack, 2000). In the epidermis, for example, stem cells reside at the tips of the deep rete ridges in palm and can be located by their high surface expression of integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$. Integrins hold cells in the right place in a tissue, and loss or alteration of integrin expression ensures departure from the stem cell niche through differentiation or apoptosis (Zhu et al., 1999). In our study we examined the position and number of integrin-bright cells within prostatic acini. However, it should be noted that we used tissue from patients that would have had some degree of BPH and that the number and position of stem cells within acini may not reflect the picture in normal prostate. Integrin-bright cells were not confined to tips of acini, for example, but were randomly located throughout each acinus unlike the epidermis, where the integrin-bright cells are arranged in clusters (Jones et al., 1995); no more than one bright cell was found together in acini examined. Approximately 1% of cells were classed as integrin bright by confocal microscopy, but this number was nearer 15% by flow cytometry; however, it is difficult to compare the two systems. From the confocal images we defined α_2-integrin-bright cells as those in which the fluorescence intensity across cell borders was at least twice the average of other cells in the transect, whereas all the cells that bound to type I collagen within 5 minutes were classed as α_2-integrin bright. However, it is clear that we cannot determine the number of stem cells by quantifying expression of a single cell surface marker. For example, 40% of cells in the basal layer of the epidermis are integrin α_2 bright, yet kinetic analysis would predict that the percentage of stem cells is closer to 10% (Potten and Morris, 1988). Kaur and Li recently reported that epidermal cells that had begun to differentiate within the basal layer retain high levels of activated β_1-integrin, but downregulate $\alpha_6\beta_4$ expression (Kaur and Li, 2000). These cells retain their adhesive capacity, indicating that induction of differentiation does not correlate with decreased β_1 expression or function. By contrast, however, we found that in prostate epithelia the rapidly adherent population also express high levels of integrins α_3 and α_6. Mutagenesis studies in which a visible cell label can be produced will be required to resolve the clonal makeup of prostate epithelia as well as the spatial relationship between stem cells and their progeny.

In conclusion we have shown that cells that express high levels of the α_2-integrin subunit have properties that indicate that they are equivalent to the stem cells of the prostate as they have the potential to establish and maintain a prostate epithelium similar to that found in vivo, with associated secretory activity. These findings are in agreement with several reports that the stem cell populations of other self-renewing tissues express similar β_1-integrin molecules (Jones et al., 1995; Hirsch et al., 1996; Jacques et al., 1998). The degree of enrichment of stem cells attainable by this method will allow further fractionation and analysis of the stem cell population to identify a set of additional markers unique for prostate epithelial stem cells. A systematic evaluation of surface molecules on the stem cells will facilitate identification and purification of these cells and greatly contribute to our understanding of pathways governing proliferative regulation and differentiation. Most importantly, stem cell research will provide a foundation for therapeutic advancement in the treatment of prostate cancer.

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