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A quantitative matrigel assay for assessing repopulating capacity of prostate stem cells

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Abstract

Homeostasis of prostate tissue is maintained by stem cells, although such cells have not been well characterized. Here, we report establishment of such a method using matrigel. Matrigel containing a single-cell suspension from adult prostatic cells was subcutaneously grafted into the flank of nude mice. Prostatic duct-like structures derived from donor tissue were observed in the gel 2 weeks after transplantation. Luminal and basal cells observed in the gel expressed several markers characteristic of prostatic and/or epithelial cells. When a mixture with both EGFP-positive and negative prostate cells was transplanted, prostatic ducts consisted of either EGFP-positive or negative cells and chimeric patterns were rarely observed, suggesting that ducts were reconstituted from a single cell. Stem cell number and function were also evaluated by competition with control cells. Overall this method revealed that cells localized in the proximal portion in prostate ducts had higher reconstitution capacity than those in the distal portion. We conclude that prostate stem/progenitor cells exist and that our method is applicable to analysis of prostate stem cells, epithelial mesenchyme interactions, and prostate cancer stem cells.

Keywords: Prostate stem cell; Matrigel transplantation

The prostate is an androgen-dependent organ that undergoes repeated regeneration and involution. It is widely accepted that the prostate, which is comprised of two major cell types, luminal and basal cells, contains renewing cells that survive androgen deprivation and proliferate when androgen is restored. Under androgen deprivation, luminal cells diminish but basal cells remain, while after androgen replacement, luminal cells reappear. Thus, basal cells are thought to contain stem/progenitor cells of prostate epithelium. However, prostate stem cells are not well characterized, since there have been no methods established for in vitro or in vivo reconstitution. Transplantation of prostatic cells combined with fetal urogenital sinus (UGS) into the subrenal capsule has shown high reconstitution ability [1], but such a grafting method has not been entirely reproducible due to technical difficulties. In vitro three dimension (3D) culture systems, which have also been used to prostatic cells, are dependent on the quality of matrix and stromal cell lines [2]. Thus, there is little evidence that single prostate cells can reconstitute an entire prostate.

Here, we establish a novel method to evaluate reconstitution of the prostate using a matrigel transplantation method. Matrigel containing a single-cell suspension of adult mouse prostatic cells was subcutaneously grafted in the flank of nude mice. Two weeks later, prostatic ductal structures were reconstituted in the gel. To determine whether ducts were clonal, we undertook competition assays with prostate cells from EGFP-transgenic mice and found that a single adult prostate stem/progenitor cell reconstitutes entire ducts of prostates. Using the matrigel

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transplantation method, we showed that proximal cells of the prostate show higher reconstitution capacity than distal cells, which is consistent with previous findings.

Materials and methods

Animals. Male C57BL/6J mice and Balb/c nude mice were purchased from CLEA Japan (Tokyo, Japan; http://www.clea-japan.com/). β-Actin-derived enhanced green fluorescence protein (EGFP) transgenic mice (C57BL/6 background) were kindly provided by M. Okabe, University of Osaka, and were bred in our animal center under conditions [3]. All procedures were performed in accordance with the guidelines of Keio University.

Matrigel transplantation. Six-week-old C57BL/6J or EGFP transgenic mice were killed by carbon dioxide inhalation. Prostates were dissected, minced with a steel blade, and digested with 1.0 mg/ml collagenase (WAKO; Osaka, Japan; http://www.wako-chem.co.jp) in 10 ml DMEM, 10% FBS (Sigma; St. Louis, MO; http://www.sigmaaldrich.com) at 37 °C for 60 min with gentle agitation. Cells were sequentially passed through 18, 21, 23, and 25 gauge needles, and finally through a 44-μm cell strainer (BD Falcon; Bedford, MA; http://www.bdbioscience.com), washed twice with 20 ml DMEM of 10% FBS, resuspended in 1 ml DMEM, 10% FBS, and counted. Cells (1×10^6) were resuspended in 10 ml DMEM, 10% FBS and mixed with 200 μl Matrigel (BD Bioscience; Bedford, MA; http://www.bdbioscience.com). This mixture was injected subcutaneously in the flank of nude mice using a 25-gauge sterile needle.

Immunohistochemical staining. Grafts were fixed in 10% neutral buffered formalin overnight and embedded in paraffin. Sections were stained with hematoxylin–eosin. For PAS staining, sections were placed in Schiff reagent for 15 min and then treated with hematoxylin for nuclear staining. For immunohistochemical staining, serial 4 μm-thick sections were deparaffinized. Antigen was retrieved by steam heating in 0.01 M citrate buffer (pH 6.0) at 121 °C for 20 min in an autoclave for GFP, E-cadherin, β-catenin, androgen receptor, SMA, K5, and K8/18 or by 4 mg/ml pepsin 0.2 N HCl at 37 °C for 20 min for ZO-1. Nonspecific binding was blocked with protein block serum Dako Cytomation; Kyoto, Japan; http:www.dakocytomation.jp, and whole-tissue sections were incubated overnight at 4 °C with polyclonal rabbit anti-EGFP (1:200; Molecular Probes; Eugene, OR; http://probes.invitrogen.com/), polyclonal rabbit anti-androgen receptor (1:200; Santa Cruz Biotechnology; Santa Cruz, CA; http://www.scbt.com/), monoclonal mouse anti-E-Cadherin (1:300; Pharmingen; San Diego, CA; http://www.bdbioscience.com/.pharmingen), monoclonal mouse anti-β-catenin (1:300; Pharmingen), polyclonal rabbit anti-K5 (1:1000; BAbCO; Richmond, CA; http://www.CRP-inc.com), polyclonal guinea pig anti-K8/18 (1:50; Progen Biotechnics, Heidelberg, Germany), polyclonal rabbit anti-ZO-1 (1:100; Zymed Laboratories, San Francisco, CA; http://www.zymed.com/index_ntscp.html), or monoclonal mouse anti-SMA FITC-conjugated clone 1A4 (1:250; Sigma). After washing with PBS, Alexa Fluor 488 goat anti-rabbit IgG or anti-guinea pig IgG, or anti-mouse IgG, or Alexa Fluor 546 goat anti-rabbit IgG (1:200; Molecular Probes) was incubated for 2 h at room temperature. For nuclear staining, specimens were treated with TOTO-3 (1:500; Molecular Probes) at room temperature for 30 min. Images were obtained using a confocal laser scanning microscope system (FV1000, Olympus; Tokyo, Japan; http://www.olympus.com).

Competitive reconstitution assay of prostate. Prostate cells from wild type or EGFP transgenic mice were prepared as single cell suspensions as described above, mixed in various proportions, and grafted. After 3 weeks, grafts were analyzed by immunohistochemistry. To compare reconstitution ability of proximal and distal prostate, 4×10^5 proximal or distal prostate cells from EGFP transgenic mice were grafted with 1×10^6 cells from wild type mice. EGFP-positive ducts in 30 ducts in each graft were counted, and experiments were repeated six times.

Statistical analysis. All results are presented as means ± the standard error. Comparisons between experimental and control groups were made using Student's *t* test.

Results

Prostate single cell suspensions can reconstitute a whole prostate duct. To establish an in vivo reconstitution assay system of the prostate gland from single cells, we performed Matrigel transplantation. Prostate lobes were obtained from 6-week-old mice under a dissecting microscope. After collagenase and a 44-μm cell strainer treatment, prostate cells were suspended as single cells (Fig. 1). In contrast, cell suspensions passed through only a 550-μm needle contained aggregation. To supply scaffold and growth factors, 1×10^6 cells were embedded in 200 μl Matrigel at 4 °C. Sol-state Matrigel was injected subcutaneously into nude mice and became gel-state at body temperature. To observe development of xenografts, tissues were analyzed at 5, 14, and 28 days post-transplantation in tissue sections. At day 5, proliferating cell clusters were observed. At day14, cell clusters showed ductal structures

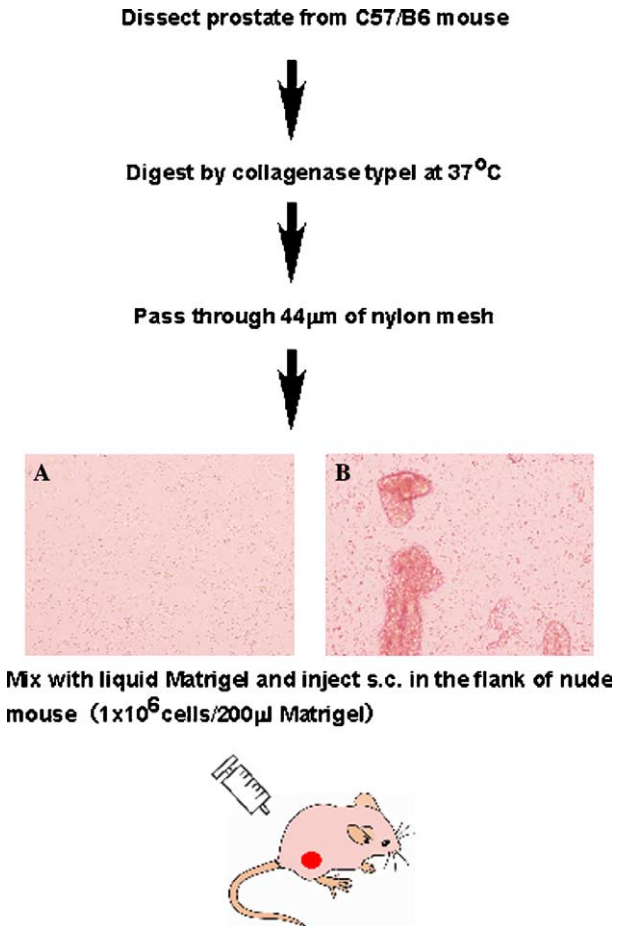

Fig. 1. Method for matrigel transplantation. To establish an in vivo reconstitution assay system of prostate from single cells, we undertook a Matrigel transplantation method. Prostate lobes were obtained from 6-week-old mice under a dissecting microscope. After collagenase and a 44 μm cell strainer treatment, prostate tissue was suspended as single cells (A). In contrast, cell suspensions passed through only a 550-μm needle contained aggregation (B). Single-cell suspension in Matrigel was injected subcutaneously into Balb/c nude mice.