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Novel In Vivo Model for Combinatorial Fluorescence Labeling in Mouse Prostate

Xiaolan Fang^{1,2}, Kenneth Gyabaah², Bitá Nickkholgh², J. Mark Cline³, and K.C. Balaji^{1,2,4,5,*}

¹Department of Cancer Biology, Comprehensive Cancer Center, Wake Forest University Health Sciences, Winston-Salem, North Carolina

²Institute for Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, North Carolina

³Department of Pathology, Section on Comparative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina

⁴Department of Urology, Wake Forest University Health Sciences, Winston-Salem, North Carolina

⁵W. G. (Bill) Hefner Veterans Administration Medical Center, Salisbury, North Carolina

Abstract

BACKGROUND—The epithelial layer of prostate glands contains several types of cells, including luminal and basal cells. Yet there is paucity of animal models to study the cellular origin of normal or neoplastic development in the prostate to facilitate the treatment of heterogeneous prostate diseases by targeting individual cell lineages.

METHODS—We developed a mouse model that expresses different types of fluorescent proteins (XFPs) specifically in prostatic cells. Using an in vivo stochastic fluorescent protein combinatorial strategy, XFP signals were expressed specifically in prostate of Protein Kinase D1 (PKD1) knock-out, K-Ras^{G12D} knock-in, and Phosphatase and tensin homolog (PTEN) and PKD1 double knock-out mice under the control of PB-Cre promoter.

RESULTS—In vivo XFP signals were observed in prostate of PKD1 knock-out, K-Ras^{G12D} knock-in, and PTEN PKD1 double knock-out mice, which developed normal, hyperplastic, and neoplastic prostate, respectively. The patchy expression pattern of XFPs in neoplasia tissue indicated the clonal origin of cancer cells in the prostate.

CONCLUSIONS—The transgenic mouse models demonstrate combinatorial fluorescent protein expression in normal and cancerous prostatic tissues. This novel prostate-specific fluorescent labeled mouse model, which we named Prorainbow, could be useful in studying benign and malignant pathology of prostate.

*Correspondence to: K. C. Balaji, Department of Urology, Cancer Biology and Institute for Regenerative Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157. kbalaji@wakehealth.edu.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Keywords

prostate; prostate hyperplasia; prostate neoplasia; proraibow; in vivo fluorescent labeling

INTRODUCTION

A healthy human prostate gland is lined up with an epithelial layer mainly composed of columnar luminal cells, basal cells, and extremely rare neuroendocrine cells, supported by surrounding stromal cells. To understand normal prostate growth, regeneration, and prostate cancer development which all involve various types of cells, it is important to dissect the cellular components in each process. While normal, hyperplastic or neoplastic prostate tissues are well characterized by morphometric studies and pathological evidence, little is known about the cellular origin of benign prostate glands in development, and there have been debates over the cell of origin in prostate cancer for decades.

Benign prostatic hyperplasia (BPH), for example, is a mixture of glandular and stromal hyperplasia associated with aging, and it affects almost every elderly man. Normal human prostate consists of about 50% glands by volume [1] and in patients with BPH the stroma to epithelia ratio is increased from 2.5:1 to 4:1 [2]. While BPH is a multifactorial disease under the influence of hormones, aging, and other microenvironment regulators, it is unclear how much of the pathology is due to genetics arising from cell of origin. Current treatments of BPH have targeted both stromal and glandular components with α -blockers and 5- α reductase inhibitors, respectively, and response to medical or surgical treatment of BPH is often varied, demonstrating a need for further understanding to promote personalized treatment for patients.

In prostate cancer, different clones of cells or monoclonal cells with varying genetic instability contributed to the heterogeneity [3-5]. This is exploited clinically by using the Gleason grading system, which is based on the two most common tumor patterns in the biopsy samples from the prostate [6]. However, it is unclear whether cancer cells progress through increasing and more aggressive grades from normal or hyperplastic cells, or arise de novo at a specific grade. Resolution of this uncertainty at cellular level could have significant impact on clinical management of patients. In prostate cancer patients, most of the cancer cells exhibit luminal cell markers and in fact, loss of basal cell makers is considered as characteristic of cancer [7-16]. In addition, there is experimental evidence suggesting that prostate cancer may originate from luminal cells. Wang et al. [17,18] reported that a population of rare luminal cells (Castration resistant NKX3.1 expressing cells, or CARNs) has stem cell potential in prostate regeneration and could give rise to prostate cancer, and recently demonstrated that luminal cells were favored as the cell of origin in the progression of prostate cancer. Compared to luminal cells, basal cells show more regenerative potential, as they express proteins involved in stem cell maintenance and differentiation (such as p63) [19] and regulator proteins which promote cell survival and invasive growth (such as Bcl-2 and C-met) [20]. Another report showed that basal cells from benign human prostate tissue were able to initiate prostate cancer in immune-deficient mice

[21]. Therefore, there is evidence in the literature supporting origin of prostate cancer from both luminal and basal cells.

In order to facilitate further understanding of the development of normal prostate gland, benign hyperplastic tissues as well as the cellular origin of prostate cancer, we used in vivo stochastic fluorescent protein combinatorial strategy to develop a mouse model that expresses specific types of fluorescent proteins in prostatic cells. This was accomplished by using the Brainbow construct, which was originally developed to distinguish adjacent neurons and visualize cellular interactions in mouse brain [22]. The Brainbow system used combination of different colors, with four basic colors in Brainbow 2.1 construct resulting in more than 100 possible combinatory signals with increasing copy numbers. We generated prostate-specific labeling model by expressing the Brainbow constructs under prostate specific promoter (rat Probasin promoter) and generated the Prorainbow mouse. To validate the utility of Prorainbow mouse model in a variety of pathological conditions in prostate, we expressed the fluorescent signals in normal, benign hyperplasia-, and neoplasia-bearing prostates.

MATERIALS AND METHODS

Mouse Strains

PB-Cre4 animals were obtained from NCI Mouse Repository (strain # 01XF5). *Gt(ROSA)26Sor^{tm1}(CAG-Brainbow2.1) Cle/J* and *129S/Sv-Kras^{tm4Tyj}/J* mice were obtained from JaxMice (stock 013731, 008180, respectively). PKD1^{lox/lox} mouse line is a generous gift from Dr. Eric N Olson's lab at University of Texas Southwestern. PTEN^{lox/lox} mouse line is kindly provided by Dr. George Kulik at Wake Forest University School of Medicine. Mouse anesthesia was done by 3–5% isoflurane inhalation and maintained up to 10 min. Mouse euthanasia was done by CO₂ inhalation followed by cervical dislocation (secondary method). All the mouse work was detailed in Animal Protocol A11-097 “Tracking Origins of Prostate Cancer” approved by the Institutional Animal Care and Use Committee (IACUC) at the Wake Forest University School of Medicine and in Animal Protocol PC094003 “Tracking Origins of Prostate Cancer: An Innovative In Vivo Modeling” approved by USAMRMC Animal Care and Use Review Office (ACURO).

Animal Tissue Preservation and Processing

Mouse tissues were harvested from euthanized male animals and fixed in 4% paraformaldehyde at 4°C overnight, and switched to 30% sucrose (in PBS) overnight. Then the tissues were embedded in Tissue-Tek O.C.T. embedding compound (Sakura Finetek, #4583) and were quickly frozen in liquid nitrogen. The samples were stored and sectioned at –20°C. 5- and 10-µm frozen sections were imaged and analyzed later.

Microscopy

For in vivo fluorescence detection, samples were imaged with a Zeiss LSM 710 laser scanning confocal microscope with 405, 488, 514, and 561 nm laser lines. All micrographs were acquired as 8-bit images at 512 × 512 or 1024 × 1024 pixels using 20× (0.8 NA) and 40× (0.95 NA) Plan-Apochromat air objectives. were used to avoid channel bleed-through.

Confocal image acquisition and analysis were controlled using Zen software. All the work was done at the Microscopic Imaging Core Facility at Wake Forest University. For regular bright field imaging, samples were imaged with a Leica DM4000B automated Upright microscope at Imaging Core Facility at Wake Forest Institute for Regenerative Medicine.

Immunofluorescence and Immunohistochemistry

For Ki-67 staining, rabbit-anti-Ki-67 antibody (Thermo Scientific, Cat# PA5-19462) was used as primary antibody and goat-anti-rabbit IgG 488 antibody (Invitrogen A11-070) as secondary antibody. For PKD1 detection, PKC μ Antibody (C-20) (Santa Cruz, Cat# sc-639) was used as primary antibody and biotinylated Goat Anti-Rabbit IgG Antibody (Vector Laboratories, Cat#BA-1000) as secondary antibody.

PCR, RT-PCR, and qPCR

Primers P021 (5'-CTGAAGAATGGGACAGGCATTG-3') and C031 (5'-CATCACTCGTTGCATCGACC-3') were used for Pb-Cre genotyping. Primers 11341 (5'-GAATTAATTCCGGTATAACTTCG-3'), oIMR8545 (5'-AAAGTCGCTCTGAGTTGTTAT-3'), and oIMR8916 (5'-CCAGATGACTACCTATCCTC-3') were used for Brainbow 2.1 transgene genotyping. For RT-PCR of PKD1 mRNA check, primers PKD1-#1 (5'-AAGTGACCATCAATGGAG-3'), and PKD1-#4 rev (5'-ACCAGGGT GATGAAGGTT-3') were used. For qPCR of PKD1 mRNA check, TaqMan Gene Expression Assay SM (Life Technologies, catalog# 4331182) was used.

Primary Prostate Cell Isolation and Flow Cytometry Analysis

The primary mouse prostate cells were collected by digestion with 1 mg/ml collagenase I & IV (Worthington Biomedical Corporation, LS004196 and LS004188) at 37°C for 2 hr with rocking. The cells were then prepared as described by Lukacs et al. [23] and passed through a 40 μ m cell strainer (BD Falcon Cat#352340) to ensure a single cell suspension. After counting the cells, they were spun down and re-suspended in 400 μ l DMEM complete medium at 1×10^6 cells/tube and transferred to the FACS machine (FACS Aria II SORP, Serial # P69500064, Becton–Dickinson, Franklin Lakes, NJ). The sorting results were analyzed by FACSDiva (version 6.1.1).

RESULTS

Prorainbow Fluorescent Labeling in Normal Prostate Gland

In order to label prostate cells specifically, the Brainbow 2.1 constructs were expressed under the prostate specific rat Probasin promoter control with the lox-Cre system. By crossing ROSA-Brainbow 2.1 mice with PB-Cre4 animals, PB-Cre4; CMV-XFP (Brainbow 2.1) pups were produced (Fig. 1). The expression of fluorescent signals in prostate was observed, and the normal prostate cells were labeled specifically (Fig. 2A). We observed expression of XFPs in all the lobes of the prostate gland (data not shown). No XFP signal was detected in other organs (lung, kidney, or liver, Supplementary Fig. S1), which confirmed the specificity of fluorescent signal expression in prostate. RFP, GFP, CFP, and YFP showed different expression ratio in normal prostate gland (Table I), and their

expression was at specific subcellular localization (RFP and YFP in cytoplasm; GFP in nucleus; CFP at cell membrane) (Fig. 2A). We also confirmed that the primary mouse prostate cells with XFP expression could be sorted using FACS and cultured in vitro for at least four days.

Prorainbow Fluorescent Labeling in Prostate With Benign and Malignant Pathology

In order to explore the utility of Prorainbow mouse models in studying different prostate pathology, we selected three transgenic mouse models with normal (PKD1 knock-out), benign hyperplasia (Kras^{G12D} knock-in), and cancer prostate (PKD1 PTEN double knock-out). Protein kinase D1 (PKD1) is a serine/threonine kinase and is essential for development, as whole body knock-out of PKD1 resulted in embryonic lethality [24]. PKD1 is down-regulated in advanced prostate, breast, and gastric cancers. We had previously shown that PKD1 suppresses EMT through phosphorylation of downstream transcription factor Snail, and inhibits cell proliferation, tumor growth, and metastasis [25,26]. We generated PKD1 knock-out Prorainbow animals (with genotype of PB-Cre4; PKD1^{lox/lox}; CMV-XFP/+ or CMV-XFP/CMV-XFP). PKD1 knock-out efficiency was confirmed at mRNA and protein level (Fig. 3A–C). In these prostate-specific PKD1 knock-out animals, we did not observe any apparent difference in prostate size or pathological phenotype compared to littermate controls up to 20 months of age (Fig. 4A and B). The prostate tissue was labeled with different endogenous fluorescent colors in PKD1 knock-out prostate (Fig. 4C).

RAS gene family consists three functional oncogenes that are often mutated in several types of cancers [27]. Ras proteins are small GTPase involved in essential signaling transduction events, and overexpression of mutated Ras proteins resulted in uncontrolled cell proliferation and cancer [28,29]. K-Ras is one of the three widely studied Ras proteins (the other two are H-Ras and N-Ras), and over-expression of K-Ras^{G12D} mutant protein was reported to promote lung cancer development when induced by lysozyme M-cre in mouse [30]. Though the mutation of K-Ras is infrequent in American patients, it is reported that in a Japanese prostate cancer patient cohort study, 27% of the patients had K-Ras mutation at codon 12 [31,32]. In the mouse model from our study, prostate-specific over-expression of K-Ras^{G12D} resulted in prostatic hyperplasia (Fig. 5A and B). With Prorainbow labeling, we observed fluorescent signals in both epithelial and stromal cells (Fig. 5C and D).

To study Prorainbow expression in prostate cancer-bearing tissues, we brought in PTEN mutations into our PKD1 KO model, by crossing PTEN KO mice with PKD1 KO Prorainbow 2.1 mice to generate PKD1 PTEN double knock-out animals (PB-Cre4; PKD1^{lox/lox}; PTEN^{lox/lox}; CMV-XFP/+). The *PTEN* (phosphatase and tensin homolog) tumor suppressor gene is one of the most frequently mutated genes in human cancer. It is reported that knock-out of *PTEN* specifically in mouse prostate recapitulates the disease progression seen in humans [33,34]. We observed significant increase in size and development of prostate cancer in PKD1 PTEN double knock-out animals (Fig. 6A and B), while the other organs (kidney, liver, and lung) did not form tumors (Supplementary Fig. S2). A thorough survey of the double knock-out animals did not demonstrate any evidence of distant metastasis up to 8 months.

While PKD1 knock-out Prorainbow mice with normal phenotype expressed XFP proteins in almost every epithelial cell, the expression of XFPs in K-Ras^{G12D} knock-in or PKD1 PTEN double knock-out Prorainbow mice was heterogenous, as indicated by the patchy color distribution (Fig. 6C), suggesting varying clones contribute to hyperplasia or prostate cancer development in those two mouse model. This finding may be exploited in understanding the origin prostate cancer as varying clones of cells, either differentially labeled or losing XFP expression in each lineage. Another possible explanation of lack of uniform XFP expression might be related to cell proliferation, as actively proliferating cells might express XFP differently compared to quiescent cells. To verify whether expression of XFP is dependent on cell proliferation, we stained the tissue with proliferation marker Ki67. No correlation was found between Ki67 and XFP expression (Fig. 6D), suggesting that XFP expression is most likely related to clonal origin of cells rather than proliferative capacity. This finding makes the model extremely valuable to study the clonal origins of primary prostate cancer, and perhaps cells at metastatic sites as well as metastatic cell might be traced back to their cell(s) of origin based on the specific fluorescent labeling of the entire cell lineage. Interestingly, we found that RFP showed a dominant expression pattern in the PKD1 PTEN double KO prostate tissues in all the lobes except ventral (Table II).

In our Prorainbow models, we verified the XFP expression in basal and luminal cells by double staining with cell type specific markers (CRK5 for basal cells and CRK8 for luminal cells) (Supplementary Fig. S3A and B). Besides epithelial cells, we also observed fluorescent protein expression in stromal cells in the prostate (Fig. 2B, Supplementary Fig. S3C). As Probasin expression was reported to be detected in epithelial cells but not in stromal cells [35], it remains possible that the stromal XFP signals we observed might be non-specific expression.

DISCUSSION

Most studies in prostate development to date have focused on ductal development, interaction with urogenital sinus, and epithelial stromal interactions [36-40]. More recent studies have focused on stem cells as a source of BPH and cancer of prostate in adults [41-47]. However, what is lacking is the exact cellular origin of prostate glands during development and fate of the glandular progenitor cells through pre-pubertal, pubertal, early, and late adulthood. Unlike other human organs, which either cease to grow or shrink in size with aging, prostate continues to enlarge. This common observation suggests that there could be a genetic imprint in prostate epithelial and stromal cells that predisposes them to growth, suppresses apoptosis, or ontogenetically reawakens them from dormancy (which possibly takes place in pre-puberty and mid-adulthood).

To understand the mechanism of BPH, we need to identify and focus at cellular level on drivers of growth. In K-Ras^{G12D} knock-in Prorainbow mouse, we observed clonal expression pattern of fluorescent signals in hyperplastic tissues (Fig. 5C and D), indicating heterogeneity in BPH. Prorainbow model could be used to focus on the study of prostate glandular development at a cellular level, which would provide novel and necessary insights in our understanding of prostate development and allow us to better manage prostatic diseases. Since our current model used Probasin promoter, which is prostate specific but not

basal or luminal cell specific, co-staining of basal or luminal cell specific markers is necessary to delineate the cell type. However, the concept of Prorainbow mouse modeling is amenable to cell type labeling as well, such as inducing fluorescent expression under cell-type specific promoters (e.g. CRK5 promoter for basal cells, CRK8 promoter for luminal cells), so as to label and trace the specific cell type more precisely. We expect Prorainbow model to be a valuable tool for cell lineage tracing in both normal and malignant prostatic development, such as prostate tissue regeneration after castration/Testosterone replacement, cancer relapse after androgen depletion, etc.

Unlike other common human cancers such as breast or lung, prostate cancer is multifocal disease, which could be of varying Gleason grade. One central question in prostate cancer progression is whether these multifocal tumors have a common origin and disseminated within the prostate gland or they arise from individual clones. The Prorainbow model could be used to answer the question. Multifocal cancers with common ancestral clones would exhibit the same fluorescent colors, whereas different colors would be seen if foci are of varying origins. Furthermore, it could be used to understand where the metastasis originates from heterogenous primary prostate tumors. Haffner et al. [4] used whole genome sequencing and molecular pathological analysis to track the evolution of the clonal origin of metastatic tumors from the prostate in the same patient, and found that the lethal clone originated from a small, low-grade cancer focus instead of the larger, high-grade primary cancer or the local lymph node metastasis. While similar studies consisting of large number of patients might be laborious, expensive, and time consuming, the Prorainbow mouse model could be used to further detail and validate findings from patients with prostate cancer. Using established mouse models for metastatic prostate cancer [48,49], we can trace the origin of metastatic cells based on the same fluorescent color, study the correlation between prostate cancer metastasis and its cell of origin, and identify whether different cellular origins would affect the metastatic potential of each cell lineage. Comparison studies of genetic alterations between metastatic cells and primary cancer cells could be done as well. The Prorainbow model could serve as a straightforward and powerful tool to sort the cells originating from the same clone/focus but disseminated at different spatial locations either within the primary tumor or at distant sites.

Another unanswered question in prostate cancer research is whether varying Gleason grades arise de novo as cancer cells progress through increasing Gleason grades. While the Gleason grading system cannot be directly applied to mouse, the Prorainbow model could be used for the issue by tracing the fluorescent proteins between multifocal tumors of similar or varying grades. Concordance of combinatorial colors between multifocal cancers of similar grade may suggest a common clonal origin. If combinatorial concordance is demonstrable between different grades, the data would suggest that tumors have progressed through the varying grades. If common colors are not decipherable, possible independent origins of varying grades of tumor could be considered as plausible explanation.

In this study, we demonstrated that stochastic expression of fluorescent colors could last for generations of cells. It is logical to assume that circulating tumor cells (CTC) arising from primary tumors would continue to express the fluorescent colors. CTC is an evolving tool in clinical management of several human cancers including prostate and has been shown to be

a reliable prognostic marker of disease progression [50-57]. The current technologies of CTC isolation relies on antibodies and magnetic beads, which are costly and not widely available. It would be much simpler to isolate cells based on fluorescent protein expression, as FACS sorting is now a commonly used laboratory technique. Combined with our data that primary prostate cells with Prorainbow signals can be separated and sorted by FACS, and that Prorainbow signals are expressed in the mouse models with aggressive prostate cancer, it is conceivable that the fluorescent labeled CTCs could be isolated from Prorainbow mouse for further cellular studies in vitro.

Prorainbow mouse might also provide abundant information of prostate development by in vivo animal imaging, which is a rapidly developing field. Existing technologies could scan depths around 500 μm to 2 mm for fluorescent signals, although image quality suffers significantly at greater depths [58]. Because prostate is situated deep in the pelvis and potential metastasis occurs in the bones and lungs, current animal in vivo imaging equipment might not be able to capture the fluorescent signals. Future developments in the field of in vivo imaging might allow for deeper penetration and detection of fluorescent proteins, in which case Prorainbow mouse model would become highly valuable for real time in vivo imaging.

CONCLUSION

The study has created and established the utility of Prorainbow mouse model to study the prostate. Because stochastic fluorescent labeling is seen fairly uniformly in benign prostate and patchier in hyperplastic and cancerous prostate, the model could be used to discriminate different pathologies in prostate in addition to studying clonal origins of cells. Concurrent development of newer technologies will increase the application of Prorainbow mouse model in animal research. The model has a potential to become a cheap and viable tool to address specific questions in cancer biology such as cellular origin of cancer and study of metastatic cascade among others.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Breeding and screening for mouse expressing fluorescent signals in normal prostate. **A:** breeding scheme. **B:** genotyping results for PB-Cre4; Brainbow2.1 lox/+ pups. Pups #15, 18, 20, 21, and 22 are positive candidates.

Fig. 2.

Fluorescent protein expression was observed in normal mouse prostate gland. **A:** frozen section of PB-Cre; Brainbow 2.1 lox/lox mouse prostate observed under confocal fluorescent microscope. As expected, RFP and YFP localize in cytoplasm, CFP on cell membrane, and GFP in nucleus (arrows). Scale bar, 50 μm . **B:** Prorainbow signals were observed in prostate stromal cells (arrows). Scale bar, 100 μm .

Fig. 3. PKD1 expression is knocked down efficiently in PKD1 KO mouse prostate. **A:** quantitative PCR, and **B:** RT-PCR for PKD1 mRNA from PKD1 KO prostate compared to litter mate control. **C:** anti-PKD1 staining by immunohistochemistry in PKD1 knock-out (KO) animal and control littermate prostate tissues.

Fig. 4. PKD1 knock-out (KO) animal showed normal prostate phenotype up to 18 months (539 days). **A:** prostate tissues harvested from PKD1 KO animal and control litter mate. No difference in sizes of the different lobes of prostate was observed. AP, anterior prostate. VP, ventral prostate. LP, lateral prostate. DP, dorsal prostate. **B:** H & E staining of both knock-out animal and control prostate tissues. **C:** fluorescent protein expression in frozen section of PKD1 KO animal with a copy of Brainbow 2.1 (PB-Cre; PKD1^{lox/lox}; Brainbow 2.1^{lox/+}) observed by confocal fluorescent microscopy. Scale bar, 100 μ m.

Fig. 5. K-Ras^{G12D} over-expressing resulted in prostate hyperplasia. **A:** prostate tissues harvested from K-Ras^{G12D} Knock-in animal. AP, anterior prostate; VP, ventral prostate; LP, lateral prostate; DP, dorsal prostate. **B:** H & E staining of K- Ras^{G12D} over-expressing prostate gland. **C** and **D**, confocal images of endogenous XFPs in K-Ras^{G12D} over-expressing prostate gland. XFPs expressed in both epithelial cells (arrowheads) and stromal cells (arrows). Scale bar, 100 µm.

Fig. 6.

PKD1 PTEN double knock-out promotes neoplasia in prostate. **A:** prostate tissues harvested from PKD1 PTEN double knock-out animal (left) and control litter mate (right). The sizes of prostate lobes were significantly increased in PTEN/PKD1 double knock-out animal. **B:** H&E staining of prostate tissues from PKD1 PTEN double knock-out animal and control litter mate. **C:** PKD1 PTEN double knock-out animals showed heterogenous XFP expression, as indicated by a few heterogenous clones with Prorainbow labeling in prostate. **D:** Prorainbow signals (RFP, red) were independent from cell proliferation in PKD1 PTEN double knock-out animal. Frozen section was stained with anti-Ki67 antibody (green) and

DAPI (blue). Arrows indicate proliferating epithelial cells (cancer cells in prostate) that were labeled with fluorescent proteins. Scale bar, 100 μm .

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TABLE I

Expression Ratio of XFPs in Normal Prostate Cells

	GFP	YFP	RFP	CFP
Total XFP-expressing cell number	17	148	285	14
Percentage	3.7%	31.9%	61.4%	3.0%
XFP-expressing cells in dorsal lobe	1	42	63	0
Percentage in dorsal lobe	0.9%	39.6%	59.4%	0.0%
XFP-expressing cells in ventral lobe	0	7	12	0
Percentage in ventral lobe	0.0%	36.8%	63.2%	0.0%
XFP-expressing cells in lateral lobe	9	58	109	6
Percentage in lateral lobe	4.9%	31.9%	59.9%	3.3%
XFP-expressing cells in anterior lobe	7	41	101	8
Percentage in anterior lobe	4.5%	26.1%	64.3%	5.1%

TABLE II

Expression Ratio of XFPs in PKD1 PTEN Double KO Prostate Cells

	GFP	YFP	RFP	CFP
Total XFP-expressing cell number	1	308	863	21
Percentage	0.1%	25.8%	72.3%	1.8%
XFP-expressing cells in dorsal lobe	0	3	73	0
Percentage in dorsal lobe	0.0%	3.9%	96.1%	0.0%
XFP-expressing cells in ventral lobe	1	4	293	9
Percentage in ventral lobe	0.3%	1.3%	95.4%	2.9%
XFP-expressing cells in lateral lobe	0	197	167	0
Percentage in lateral lobe	0.0%	54.1%	45.9%	0.0%
XFP-expressing cells in anterior lobe	0	0	200	0