



Tumor Dormancy and Slow-Cycling Cancer Cells

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Abstract

Cancer cell heterogeneity is a universal feature of human tumors and represents a significant barrier to the efficacy and duration of anticancer therapies, especially targeted therapeutics. Among the heterogeneous cancer cell populations is a subpopulation of relatively quiescent cancer cells, which are in the G0/G1 cell-cycle phase and refractory to anti-mitotic drugs that target proliferative cells. These slow-cycling cells (SCCs) preexist in untreated tumors and frequently become enriched in treatment-failed tumors, raising the possibility that these cells may mediate therapy resistance and tumor relapse. Here we review several general concepts on tumor cell heterogeneity, quiescence, and tumor dormancy. We discuss the potential relationship between SCCs and cancer stem cells (CSCs). We also present our current understanding of how SCCs and cancer dormancy might be regulated. Increasing knowledge of SCCs and tumor dormancy should lead to identification of novel molecular regulators and therapeutic targets of tumor relapse, residual diseases, and metastasis.

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Keywords

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Introduction: Cellular Heterogeneity in Cancer

Cellular heterogeneity represents an omnipresent feature in human tumors, which contain distinct subsets of cancer cells with diverse morphology, cytogenetic markers, growth kinetics, immunological characteristics, metastatic ability, and sensitivity to therapeutics [1]. Understanding cellular heterogeneity in solid tumors should facilitate development of new diagnostic and therapeutic strategies. The majority of current anti-cancer drugs are anti-mitotic and molecularly targeted agents, which mainly target the differentiated and proliferating cancer cells [2]. However, recent evidence suggests that there is always a population of quiescent, slow-cycling cells (SCCs) that preexist in primary tumors and is less affected by standard treatments. *Experimentally*, reports have shown that SCCs exist in many human cancer cell

cultures and xenograft tumors [2, 3]. *Clinically*, patients with cancer can develop recurrent and metastatic disease with latency periods that range from years to decades. *Pathologically*, subsets of Ki-67-negative cells, characterizing diminished proliferation, are always observed in virtually all human tumors [4]. These all indicate the existence of SCCs in models as well as patient tumors.

Quiescence and Slow-Cycling Cells (SCCs) in Cancer

Quiescent, slow-cycling cells (SCCs) exist in clinical human tumors [4], and accumulating evidence suggests that SCCs may play a vital role in many aspects of cancer biology. Due to their intrinsic dormancy, SCCs are thought to be insensitive to most current clinical treatments, and thus may contribute to tumor relapse [3]. However, few studies have developed systems to PROSPECTIVELY study SCCs. Upon entering the non-proliferative G0/G1 cell-cycle phase, cells have relatively low metabolic activity in a state of cell-cycle arrest, a term referred to as quiescence. Transforming growth factor- β (TGF- β) is among the many secreted factors that mediate microenvironmental signaling that controls cellular differentiation, proliferation, and survival. TGF- β has been shown to have tumor suppressive properties in that it opposes normal epithelial cell proliferation [5]. TGF- β has been reported to maintain dormancy in stem cell populations in the prostate [6], liver [7], gastrointestinal system [8], and in the bone marrow [9].

In many cancers, a small population of SCCs has been identified in cell cultures, xenografts, and clinical tumors, and SCCs are thought to survive anticancer therapies and contribute to later disease recurrence and metastasis [2–4, 10]. Additionally, SCCs may also have implications in immunotherapy; for example, vaccination with drug-resistant slow-cycling tumor cells caused a reduction in tumor volume and prolonged the overall survival of tumor-bearing mice [11]. Therefore, isolation and functional study of SCCs will be keys to developing future therapies that

better target dormant cancer cells to prevent recurrence.

Clinical Evidence for Cancer Dormancy

Although poorly understood, cancer dormancy has been generally classified into two entities: (1) tumor mass dormancy, whereby active cancer cell proliferation is mechanistically opposed by apoptosis, and (2) tumor cell dormancy, characterized by inactive tumor cells that have entered into prolonged G0/G1 cell-cycle arrest [4]. Whereas tumor mass dormancy is limited by factors such as poor neovascularization and susceptibility to immune surveillance, tumor cell dormancy represents a clinically asymptomatic form of dormancy where these cells can become active months to decades later. The major concern of dormant cancer cells is whether they can be revived from an inert state to rapidly growing overt deadly cancers. This raises the question of how to detect dormant cells. The evidence for dormant cancers has been demonstrated by autopsies of patients diagnosed with cancer as well as trauma victims [12]. Other evidence of cancer cell dormancy arises from the findings that disseminated tumor cells (DTCs), which are cells that physically separate from the primary tumor mass and travel to other sites in the body via circulation, have the ability to enter a dormant state and become resistant to therapies [13].

Cancer Stem Cells (CSCs)

The topic of CSCs has been debated by researchers throughout the years mostly because of the lack of the ability to consistently assay and uniformly define these cell populations. Stem cells (SCs) are defined as cells that possess self-renewal and differentiation abilities [14]. Historically, most SCs in adult tissues and organs have been identified by panels of cell surface markers, in vivo lineage tracing, and by their intrinsic quiescent and slow-cycling properties [3]. Additionally, functional assays that measure

drug efflux (e.g., side population) and aldehyde dehydrogenase (ALDH) detoxifying capacity (i.e., Aldefluor) may be used to purify and enrich stem cells [1]. CSC is a functional term and can be most properly defined in functional assays by their ability to re-generate serially transplantable tumors with features of the parent tumor (e.g., cellular heterogeneity and specific cell surface markers) [3]. Several terms, including dormant or quiescent cells, SCCs, and label-retaining cells (LRCs) may be used interchangeably; however, CSCs may not necessarily be included among these cell types. Generally, dormant or residual slow-cycling tumor cells are thought to be a major source of tumor relapse and metastasis, and are therefore an obstacle to therapy. SCCs and CSCs are two “semantic” terms describing two overlapping cancer cell subpopulations in a continuum [2–4]. In other words, some (but NOT all) SCCs may possess both phenotypic and functional properties of CSCs and vice versa, and some (but NOT all) CSCs may be dormant and slow-cycling. For instance, SCCs in some tumors have been shown to possess CSC-related properties, e.g., enhanced tumor-propagating ability, therapy resistance, and promotion of tumor relapse and metastasis [3]. On the other hand, although some CSCs, e.g., the prostate-specific antigen-negative/low (PSA^{-/lo}) CSCs [1] and the cluster of differentiation 44-positive (CD44⁺) [15, 16] prostate cancer (PCa) cell populations, are relatively quiescent at the population level, other CSC populations, e.g., the ALDH⁺ cells [1, 3], may be proliferative. This is analogous to the existence of well-established quiescent AND cycling normal SC populations [3]. Much more effort has been devoted to the studies of CSCs than SCCs, as evidenced by identification of CSCs in virtually every tumor system [3, 16–20]. However, SCCs clearly exist in tumors and may play a critical role in regulating tumor cell subpopulation dynamics. For example, a population of slow-cycling melanoma cells bearing a lysine demethylase 5B-high (KDM5B-high) phenotype is required for continuous tumor growth [21], and therapeutically targeting this population can overcome the intrinsic multidrug resistance in melanoma [22]. Our lab is currently cross-

examining the inter-relationship between purified PCa SCCs and prostate cancer stem cells (PCSCs) identified using the PSA^{-/lo} phenotype and other markers such as CD44⁺, ALDH-high, and ABCG2⁺ to compare their relative “stemness,” aggressiveness, therapy sensitivities, and ability to repopulate recurrent tumors.

Modeling Cancer Cell Dormancy

A recent review by Kester and van Oudenaarden discussed new advances in sequencing technologies used either alone or in combination to predict cellular differentiation trajectories based on single-cell transcriptomics [23]. For example, the techniques described combines single-cell genetic lineage tracing with differentiation trajectory algorithms to reliably capture cell-type heterogeneity to therapy responses. These same technologies can be used to investigate lineage relationship between stem cells and their mature progeny, traced over time. Tracking lineage-related changes in genomic signatures by either introducing specific alterations experimentally (prospective lineage tracing) or following intrinsic non-perturbation processes (retrospective lineage tracing), researchers can differentially determine gene signatures associated with a single founder cell [23]. The utility of dyes into single founder cells along with flow-assisted cell sorting (FACS) has opened many avenues for investigators to more readily study cancer cell initiation, cell-of-origin, and tumor dormancy.

Studies have also been utilizing high-throughput single-cell RNA-Seq (scRNA-Seq) to investigate cellular heterogeneity as progression trajectory in relation to metabolomic changes that occur during chemical reprogramming [24]. Such novel powerful techniques demonstrate that fully differentiated, mature cell progression tracing can be associated with transcriptomic and epigenomic changes that allow cell fate experimental manipulations to be performed prospectively.

Yet another powerful method of studying tumor dormancy is to employ the LRC model. Generally, normal mammalian adult SCs are

slow-cycling, and long-lived cycling SCs have been reported in rapidly renewing tissues such as hair follicles, small intestine, and blood [25]. In practice, label-retaining techniques are frequently employed to study SCs, although this technique is meant to label SCCs rather than SCs. In this technique, tissues (or cells) are first pulsed with a DNA base analog, e.g., 5-bromo-2'-deoxyuridine (BrdU), which is followed by an extended period of chase. Such identified LRC population is often enriched in functional SCs. In addition to DNA analogs, which unfortunately CANNOT be used to purify out LIVE SCCs, other label-retaining techniques (e.g., Tet-controlled H2B-GFP fusion protein and cell membrane labeling dyes such as PKH26) have been developed to identify and purify SCCs for functional studies [26].

Visualizing cell cycle transitions, generally, has proved difficult. A relatively new technique known as the FUCCI (fluorescence ubiquitination cell-cycle indicator) has been developed, which exploits the inversely oscillating levels of two separate cell-cycle licensing factors, fused with either green or red fluorescent probes [27]. In this system, time-lapse video fluorescence microscopy captures the switch from green- to red-emitting signals as the cell cycles from G1 to S/G2/M, respectively [27]. Modified FUCCI-based systems allow for G0/G1-phase cell separation to study dormancy in FUCCI-expressing cell lines, stem cell lineages, and in mouse models [28], allowing the enrichment and isolation of SCCs for studying their involvement in therapy resistance, disease recurrence, and drug screening.

Recently developed 3D cell and tissue culture technologies such as organoid systems have become increasingly efficient in drug development and personalized medicine for primary and metastatic colorectal, pancreatic, prostate, breast, and brain tumors [29]. Organoids are currently used to model mutational processes underlying tumorigenesis, response to immunotherapies, and the contribution of CSCs to tumor growth.

Pharmacokinetic and pharmacodynamic (PK/PD) methods have recently incorporated imaging techniques to assess the delivery and efficacy of fluorescently labeled drugs by in vivo micros-

copy, e.g., to investigate tumor-stromal signaling, tumor vasculature, and drug efflux capacity of CSCs [30]. Dynamically tracking circulating tumor cells (CTCs) found in the bloodstream has led to the introduction of “real-time” liquid biopsies, a way to routinely monitor cancer progression, relapse, and patient response to therapies with minimal invasion and low risk for effects [31].

Prostate Cancer as a Model of Cancer Dormancy-Related Drug Resistance

Prostate cancer (PCa) is a heterogeneous malignancy. Androgen deprivation therapy (ADT) is the current main therapeutic regimen for advanced PCa patients. However, most treated patients invariably develop the castration-resistant prostate cancer (CRPC). The cell(s)-of-origin for and mechanisms underlying CRPC development and maintenance remain poorly understood. Recently, we have reported a PSA^{-lo} PCa cell population that exists in primary tumors at low frequency but dramatically increases in high-grade primary tumors and, in particular, recurrent PCa [15]. Importantly, the PSA^{-lo} population, which expresses stem cell gene signatures and possesses many cardinal SC properties, can function as both cells-of-origin AND tumor-propagating cells in CRPC [1, 15, 32]. The PSA^{-lo} PCa cell population, compared to PSA⁺ cells, is quiescent, enriched for CSCs that express low androgen receptor (AR), and drug-resistant and tumorigenic [1, 15, 33]. In multiple other cancer systems, studies have also suggested that SCCs can survive anticancer therapies and contribute to later disease progression and metastasis [2–4, 21, 34]. However, no prospective studies have been performed to elucidate the clinical importance of SCCs in PCa, especially in response to ADT and subsequent CRPC development. Therefore, utilizing PCa as a model disease system, we aim to describe the implications of PCa SCCs in more broad terms of cellular quiescence and tumor cell dormancy and apply these concepts

to other cancer types as it relates to therapy resistance and repopulation of primary cancers, disease recurrence, and progression to invasion and metastasis.

Unfortunately, to our knowledge, no systematic and prospective studies have focused on SCCs as potential effectors and mediators of therapy (including castration) resistance in PCa and emergence of CRPC. Moreover, no drugs have been developed as yet to specifically target quiescent PCa cells. As in other tumor systems, there is an urgent need to advance our knowledge of quiescent cell biology and thus provide a strong foundation to develop potential therapeutic strategies to target this obstinate population. One example would be to develop novel treatment options by combining ADT and SCC-specific therapies to eventually prevent/eliminate CRPC in patients, which should ultimately impact PCa patient survival.

LRCs and Normal Mouse and Human Prostate SCs

Adult prostate renews slowly and can undergo multiple rounds of castration-induced regression and testosterone-induced regrowth, attesting the presence and the functional importance of SCs. Since the first report of putative prostate SCs via LRC experiments by Dr. E. Wilson's group, the proximal region (i.e., close to the urethra) of the prostatic tubules [35] and the basal location [36–40] have been proposed to be the niche to maintain the quiescence (mediated by TGF- β) [6] of prostate SCs. By using lineage-tracing techniques, studies have reported the existence of lineage-restricted stem/progenitor cells within both basal and luminal layers of the mouse prostate [41–43], which is further validated by a recently developed 3D organoid system in the human prostate [44]. Importantly, these reports indicate that primitive prostate SCs are generally quiescent in vivo [41, 45]. Our lab, over the years, has studied, and made extensive use of, normal primary human prostate epithelial cells from normal/benign human prostates [1, 16, 46–48]. We recently described

a genome-wide transcriptome analysis of human prostatic basal and luminal populations using deep RNA-Seq, and found that basal cells are generally quiescent in situ and molecularly resemble aggressive PCa [47]. Also, we recently developed a feasible 2D culture system to enrich high numbers of human prostate luminal progenitor cells and further showed that these cells could function as a cell-of-origin for PCa [46]. In addition, we have established several complementary experimental strategies that enable us to purify out LIVE SCCs from human PCa cell cultures and xenograft tumors for probing their functional properties. Importantly, we have also generated unique transgenic mouse models of label-retaining cells (LRCs) to study SCCs in a naïve tumor microenvironment under unperturbed and androgen-ablated conditions. The utility of these techniques will help in determining the inter-relationship between SCCs and CSCs as well as the expression status of the AR in the SCC population and its impact on SCC biology. By performing gene-expression analysis coupled with functional assays, it may be feasible to identify potential therapeutic targets that could lead to the elimination of this “hard-to-kill” population.

PCSCs (Prostate Cancer Stem Cells): Hierarchical Organization and Relative Dormancy

Over the past 15 years, our lab has been meticulously dissecting the FUNCTIONAL heterogeneity in human PCa cells. Our systematic work, which has provided a framework of understanding of PCa cell heterogeneity, has demonstrated that the human PCSC pool largely resides in the undifferentiated PSA^{-lo} PCa cell population [1, 9, 15, 16, 32, 49]. The PSA^{-lo} PCSC pool contains multiple subsets of tumorigenic cells [50–53] and, importantly, many PSA^{-lo} PCa cells and subsets of PCSCs lack appreciable expression of AR and are dormant, which, together, render these cells intrinsically refractory to both anti-androgens and anti-mitotic drugs such as docetaxel and etoposide. Whether SCCs are het-

erogeneous in AR expression and what is the impact of AR status on SCC functions have yet to be investigated. Another area of interest includes the determination of whether PCa SCCs can survive ADT and repopulation CRPC in vitro and in vivo, and characterizing transcriptome changes of LRCs during CRPC emergence.

Many molecular entities and circuits initiate and enforce cancer cell dormancy. For example, LRIG1 (leucine-rich repeats and immunoglobulin-like domains protein 1), known as a pan-ERBB negative regulator, is well established to promote adult stem cell quiescence, especially in epidermis and gastrointestinal system [15, 54–59]. LRIG1 functions as a tumor suppressor in many cancers [58]. Gene-expression profiling reveals LRIG1 enrichment in PSA^{-lo} PCa cell population [15]. The PSA^{-lo} cell population harbors highly dormant PCSCs possessing tumorigenic, metastatic, and CRPC-initiating and -propagating properties.

Of great clinical interest is whether a tumor can be contained indefinitely in a dormant, non-malignant state or whether driving SCCs out of dormancy is a better therapeutic approach. There exist several research opportunities to study the differences between persistence of tumor dormancy versus reactivation. Studies have demonstrated that CoCo, a bone morphogenic protein 4 (BMP-4) inhibitor protein secreted by the stroma [10], reactivates dormant breast cancer cells localized to the lungs, providing evidence of DTC escape from dormancy. It was also found that breast cancer cells with low CoCo expression remained dormant [60]. Thus, it is important to develop a systematic functional characterization of SCCs in human PCa cells and xenograft tumors, LRCs in genetic mouse models of prostate tumors, and gene-expression profiling of SCCs in human and mouse PCa to address the clinical implications associated with tumor dormancy.

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References

- Liu, X., et al. (2015). Systematic dissection of phenotypic, functional, and tumorigenic heterogeneity of human prostate cancer cells. *Oncotarget*, 6(27), 23959–23986.
- Moore, N., & Lyle, S. (2011). Quiescent, slow-cycling stem cell populations in cancer: A review of the evidence and discussion of significance. *Journal of Oncology*, 2011, 396076.
- Tang, D. G. (2012). Understanding cancer stem cell heterogeneity and plasticity. *Cell Research*, 22(3), 457–472.
- Aguirre-Ghiso, J. A. (2007). Models, mechanisms and clinical evidence for cancer dormancy. *Nature Reviews. Cancer*, 7(11), 834–846.
- Salm, S., Burger, P. E., & Wilson, E. L. (2012). TGF-beta and stem cell factor regulate cell proliferation in the proximal stem cell niche. *Prostate*, 72(9), 998–1005.
- Salm, S. N., et al. (2005). TGF-beta maintains dormancy of prostatic stem cells in the proximal region of ducts. *The Journal of Cell Biology*, 170(1), 81–90.
- Santoni-Rugiu, E., et al. (2005). Progenitor cells in liver regeneration: Molecular responses controlling their activation and expansion. *APMIS*, 113(11–12), 876–902.
- Mishra, L., et al. (2005). The role of TGF-β and Wnt signaling in gastrointestinal stem cells and cancer. *Oncogene*, 24(37), 5775–5789.
- Wilson, A., & Trumpp, A. (2006). Bone-marrow haematopoietic-stem-cell niches. *Nature Reviews. Immunology*, 6(2), 93–106.
- Yadav, A. S., et al. (2018). The biology and therapeutic implications of tumor dormancy and reactivation. *Frontiers in Oncology*, 8, 72.
- Sun, Q., et al. (2012). Immunotherapy using slow-cycling tumor cells prolonged overall survival of tumor-bearing mice. *BMC Medicine*, 10, 172.
- Almog, N. (2010). Molecular mechanisms underlying tumor dormancy. *Cancer Letters*, 294(2), 139–146.
- Sosa, M. S., Bragado, P., & Aguirre-Ghiso, J. A. (2014). Mechanisms of disseminated cancer cell dormancy: An awakening field. *Nature Reviews. Cancer*, 14(9), 611–622.
- Jeter, C. R., et al. (2009). Functional evidence that the self-renewal gene NANOG regulates human tumor development. *Stem Cells*, 27(5), 993–1005.
- Qin, J., et al. (2012). The PSA(−/lo) prostate cancer cell population harbors self-renewing long-term tumor-propagating cells that resist castration. *Cell Stem Cell*, 10(5), 556–569.
- Tang, D. G., et al. (2007). Prostate cancer stem/progenitor cells: Identification, characterization, and implications. *Molecular Carcinogenesis*, 46(1), 1–14.
- Bonnet, D., & Dick, J. E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Medicine*, 3(7), 730–737.

18. Collins, A. T., et al. (2005). Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Research*, 65(23), 10946–10951.
19. Singh, S. K., et al. (2003). Identification of a cancer stem cell in human brain tumors. *Cancer Research*, 63(18), 5821–5828.
20. Wright, M. H., et al. (2008). Brca1 breast tumors contain distinct CD44+/CD24- and CD133+ cells with cancer stem cell characteristics. *Breast Cancer Research*, 10(1), R10.
21. Roesch, A., et al. (2010). A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell*, 141(4), 583–594.
22. Roesch, A., et al. (2013). Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. *Cancer Cell*, 23(6), 811–825.
23. Kester, L., & van Oudenaarden, A. (2018). Single-cell transcriptomics meets lineage tracing. *Cell Stem Cell*, 23(2), 166–179.
24. Zhao, T., et al. (2018). Single-cell RNA-Seq reveals dynamic early embryonic-like programs during chemical reprogramming. *Cell Stem Cell*, 23(1), 31–45.e7.
25. Li, L., & Clevers, H. (2010). Coexistence of quiescent and active adult stem cells in mammals. *Science*, 327(5965), 542–545.
26. Zhang, D., et al. (2018). Histone 2B-GFP label-retaining prostate luminal cells possess progenitor cell properties and are intrinsically resistant to castration. *Stem Cell Reports*, 10(1), 228–242.
27. Sakaue-Sawano, A., et al. (2008). Visualizing spatio-temporal dynamics of multicellular cell-cycle progression. *Cell*, 132(3), 487–498.
28. Zielke, N., & Edgar, B. A. (2015). Fucci sensors: Powerful new tools for analysis of cell proliferation. *Wiley Interdisciplinary Reviews: Developmental Biology*, 4(5), 469–487.
29. Drost, J., & Clevers, H. (2018). Organoids in cancer research. *Nature Reviews. Cancer*, 18(7), 407–418.
30. Miller, M. A., & Weissleder, R. (2017). Imaging of anticancer drug action in single cells. *Nature Reviews. Cancer*, 17(7), 399–414.
31. van der Toom, E. E., Verdone, J. E., & Pienta, K. J. (2016). Disseminated tumor cells and dormancy in prostate cancer metastasis. *Current Opinion in Biotechnology*, 40, 9–15.
32. Chen, X., et al. (2016). Defining a population of stem-like human prostate cancer cells that can generate and propagate castration-resistant prostate cancer. *Clinical Cancer Research*, 22(17), 4505–4516.
33. Horning, A. M., et al. (2018). Single-cell RNA-seq reveals a subpopulation of prostate cancer cells with enhanced cell-cycle-related transcription and attenuated androgen response. *Cancer Research*, 78(4), 853–864.
34. Perego, M., et al. (2018). A slow-cycling subpopulation of melanoma cells with highly invasive properties. *Oncogene*, 37(3), 302–312.
35. Tsujimura, A., et al. (2002). Proximal location of mouse prostate epithelial stem cells: A model of prostatic homeostasis. *The Journal of Cell Biology*, 157(7), 1257–1265.
36. Lawson, D. A., et al. (2007). Isolation and functional characterization of murine prostate stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, 104(1), 181–186.
37. Burger, P. E., et al. (2005). Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. *Proceedings of the National Academy of Sciences of the United States of America*, 102(20), 7180–7185.
38. Lawson, D. A., et al. (2010). Basal epithelial stem cells are efficient targets for prostate cancer initiation. *Proceedings of the National Academy of Sciences of the United States of America*, 107(6), 2610–2615.
39. Xin, L., Lawson, D. A., & Witte, O. N. (2005). The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 102(19), 6942–6947.
40. Xin, L., et al. (2007). Self-renewal and multilineage differentiation in vitro from murine prostate stem cells. *Stem Cells*, 25(11), 2760–2769.
41. Wang, Z. A., et al. (2013). Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. *Nature Cell Biology*, 15(3), 274–283.
42. Choi, N., et al. (2012). Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. *Cancer Cell*, 21(2), 253–265.
43. Wang, X., et al. (2009). A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature*, 461(7263), 495–500.
44. Karthaus, W. R., et al. (2014). Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell*, 159(1), 163–175.
45. Ousset, M., et al. (2012). Multipotent and unipotent progenitors contribute to prostate postnatal development. *Nature Cell Biology*, 14(11), 1131–1138.
46. Zhang, D., et al. (2017). Developing a novel two-dimensional culture system to enrich human prostate luminal progenitors that can function as a cell of origin for prostate cancer. *Stem Cells Translational Medicine*, 6(3), 748–760.
47. Zhang, D., et al. (2016). Stem cell and neurogenic gene-expression profiles link prostate basal cells to aggressive prostate cancer. *Nature Communications*, 7, 10798.
48. Bhatia, B., et al. (2008). Critical and distinct roles of p16 and telomerase in regulating the proliferative life span of normal human prostate epithelial progenitor cells. *The Journal of Biological Chemistry*, 283(41), 27957–27972.
49. Rycaj, K., et al. (2016). Longitudinal tracking of subpopulation dynamics and molecular changes during LNCaP cell castration and identification of inhibitors

- that could target the PSA-/lo castration-resistant cells. *Oncotarget*, 7(12), 14220–14240.
50. Patrawala, L., et al. (2006). Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene*, 25(12), 1696–1708.
 51. Patrawala, L., et al. (2005). Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. *Cancer Research*, 65(14), 6207–6219.
 52. Patrawala, L., et al. (2007). Hierarchical organization of prostate cancer cells in xenograft tumors: The CD44+alpha2beta1+ cell population is enriched in tumor-initiating cells. *Cancer Research*, 67(14), 6796–6805.
 53. Patrawala, L. T., & G, D. (2007). CD44 as a functional cancer stem cell marker and a potential therapeutic target. In *Autologous and cancer stem cell gene therapy* (pp. 317–334). Singapore: World Scientific Publishing.
 54. Choi, E., et al. (2018). Lrig1+ gastric isthmal progenitor cells restore normal gastric lineage cells during damage recovery in adult mouse stomach. *Gut*, 67(9), 1595–1605.
 55. Jensen, K. B., et al. (2009). Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell*, 4(5), 427–439.
 56. Jensen, K. B., & Watt, F. M. (2006). Single-cell expression profiling of human epidermal stem and transit-amplifying cells: Lrig1 is a regulator of stem cell quiescence. *Proceedings of the National Academy of Sciences of the United States of America*, 103(32), 11958–11963.
 57. Powell, A. E., et al. (2012). The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell*, 149(1), 146–158.
 58. Wang, Y., Poulin, E. J., & Coffey, R. J. (2013). LRIG1 is a triple threat: ERBB negative regulator, intestinal stem cell marker and tumour suppressor. *British Journal of Cancer*, 108(9), 1765–1770.
 59. Wong, V. W., et al. (2012). Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nature Cell Biology*, 14(4), 401–408.
 60. Linde, N., Fluegen, G., & Aguirre-Ghiso, J. A. (2016). The relationship between dormant cancer cells and their microenvironment. *Advances in Cancer Research*, 132, 45–71.