Chapter 11
Prominin-1 (CD133) Expression in the Prostate and Prostate Cancer: A Marker for Quiescent Stem Cells

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Abstract The origin and phenotype of stem cells in human prostate cancer remains a subject of much conjecture. In this scenario, CD133 has been successfully used as a stem cell marker in both normal prostate and prostate cancer. However, cancer stem cells have been identified without the use of this marker, opening up the possibility of a CD133 negative cancer stem cell. In this chapter, we review the current literature regarding prostate cancer stem cells, with specific reference to the expression of CD133 as a stem cell marker to identify and purify stem cells in normal prostate epithelium and prostate cancer.

Keywords AC133 • Cancer stem cells • CD133 • Prominin-1 • Prostate • Prostate cancer • Stem cells

11.1 Introduction

The prostate is a small extra peritoneal gland, which sits under the bladder and in front of the rectum. The major function of the prostate is to produce a slightly alkaline fluid, which constitutes 20% of the ejaculate and contains polyamines and proteins, such as prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA). It also functions as an endocrine gland, rapidly metabolizing testosterone to the more effective dihydrotestosterone [1].

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11.1.1 Cellular Architecture of the Prostate Epithelium

Prostate tissue is organized as tubular-alveolar glands comprised of an epithelial parenchyma surrounded by fibromuscular stroma. The mature human prostate epithelium is bilayered and is mainly composed of three kinds of cells: luminal cells, basal cells, and neuroendocrine cells. Terminally differentiated luminal cells, that is, the exocrine compartment of the prostate, are the most abundant cell type in normal and hyperplastic epithelium and secrete both PSA and PAP into the glandular lumen. These cells are dependent on androgens for their survival [2] and consequently express high levels of androgen receptor (AR) [3]. The relatively undifferentiated basal cells are in direct contact with the basement membrane, which separates the epithelial and the stromal compartments. They lack secretory activity, express low or undetectable levels of AR, and are not dependent on androgens for their survival [4]. Rare neuroendocrine cells (NE) are scattered throughout the basal layer. NE cells are terminally differentiated but androgen-insensitive [5], and release neuroendocrine peptides, such as bombesin, calcitonin, and parathyroid hormone-related peptide [6], which support epithelial growth and viability.

11.1.2 Prostate Cancer

Prostate cancer (PCa) is the most commonly diagnosed cancer in men in the western world. It is generally regarded to be slow growing and originates from a pre-neoplastic lesion termed prostate intraepithelial neoplasia (PIN) [7]. At the cellular level, PCa is characterized by a drastic reduction of the basal cell content (<1% of the cells) and a concomitant expansion of the AR^+ luminal cell compartment [8], which becomes highly proliferative [9]. This change in cellular composition is accompanied by a progressive degradation of the prostatic architecture, resulting in loss of the glandular structure and destruction of the basement membrane [10, 11].

In the case of localized PCa, surgical removal of the prostate (radical prostatectomy) is still the elected therapy and is curative in the majority of cases [12], but is not without side effects. Patients with more advanced and metastatic PCa are usually treated with androgen ablation therapy, targeted toward the AR^+ secretory luminal cancer cells that constitute the bulk of the tumor [13]. Although initially PCa responds to antiandrogens, around 25% of the cases relapse and develop castration-resistant prostate cancer (CRPC) [14] with a median life expectancy of 2 years [15].

11.2 Prostate Stem Cells and the Epithelial Hierarchy

Similar to many other epithelia in the human body, there is strong evidence that homeostasis of the prostate epithelium is governed by a hierarchy of cells with different proliferative potentials [16]. In the model originally proposed by Isaacs and

Coffey [17], undifferentiated stem cells reside in the basal compartment of the prostate epithelium and give rise to terminally differentiated luminal cells through an amplifying progeny. Observations supporting this hypothesis go back to the mid-1980s, where experiments of prostate regression/regeneration in castrated rats showed the existence of long-lived, androgen-independent cells which had the ability to reconstitute a fully differentiated epithelium upon androgen reintroduction [18, 19]. Evidence linking basal to luminal cells in the same hierarchy comes also from histological studies showing the presence of intermediate cells which express basal and luminal markers [20–22], suggesting that prostate epithelial cells are in a continuum of differentiation stages within a hierarchical system. In situ lineage-tracing studies of human prostate tissues show that all the prostate epithelial cell types have a common clonal origin [23] and confirm a basal stem cell phenotype [24]. In the last decade, a solid body of evidence, using both in vitro and in vivo models, confirmed that prostate stem cells have a basal phenotype [25, 26], possess a high proliferative potential [27], can differentiate to luminal cells [28, 29], and can reconstitute prostatic-like acinar structure in vivo [27, 30]. Notably, Leong and colleagues were able to reconstitute functional prostatic structures from a single $Lin^{-}Sca\text{-}1^{+}/CD133^{+}/CD44^{+}/CD117^{+}$ cell [31].

Two independent reports however seem to contradict this hypothesis, showing the presence of a luminal progenitor in the mouse prostate. Castration-resistant Nkx3.1-expressing cells (CARNs) are rare luminal cells which (after castration) can self-renew in vivo and reconstitute prostatic ducts in a single cell transplantation experiment [32]. Moreover, lineage-tracing experiments recently showed that the basal and luminal compartments of the mouse prostate have independent stem cells, which regenerate only the compartment of origin during two consecutive rounds of castration/regeneration [33]. Both studies show evidence for an androgen-independent luminal progenitor cell that is responsible for regenerating androgen-dependent luminal cells, in contrast to the basal cell phenotype seen in humans (and in the original mouse experiment from Leong and colleagues [31]). Although of great importance, these results could reflect a difference in the prostate biology between mice and humans, as all the evidence points to a single lineage for basal, luminal, and neuroendocrine cells in the human prostate. In fact, mouse and human prostate show differences at the anatomical and cellular levels: the mouse prostate is composed of four distinct lobes, not recognizable in the human prostate, which is subdivided into zones [34]. Moreover, the mouse prostate epithelium lacks a complete basal layer, with scattered basal cells intercalated by luminal cells in direct contact with the basement membrane [35].

11.2.1 Epithelial Hierarchy in Prostate Cancer and Cancer Stem Cell Hypothesis

As discussed previously, prostate cancer is characterized by an expansion of the luminal cell compartment which has acquired the ability to proliferate [9]. However, several independent reports support the idea that a small subpopulation of aberrant

basal cells persist within PCa [36–38]. As normal prostate stem cells reside within the basal layer, the idea emerged that rare undifferentiated basal cells within PCa could contain stem-like cancer cells which are able to self-renew and generate aberrantly differentiated cancer cells. There is now a wealth of evidence that tumors are hierarchically arranged, a pattern shared between leukemias [39, 40], breast [41], brain [42], colon [43], pancreas [44], liver [45], lung [46], and endometrium [47], where cancer stem cells (CSCs) have been successfully selected using the same markers that identify the correspondent normal stem cells. In the last few years, the identification of CSCs has been one of the key research topics in prostate cancer; however, the field is still debating the precise phenotype and origin of prostate CSCs. In fact, depending on the markers, the models and the kind of assay used to test for "stemness," several groups have identified stem-like cells with both a basal and luminal origin.

The evidence that cancer stem cells reside in the luminal compartment comes from studies in transgenic mice, where luminal cells targeted with oncogenic transgenes were able efficiently to generate tumors in mice. For example, the selective deletion of PTEN (frequently mutated in human cancers) and in cells expressing PSA [48], cytokeratin 8 [33], or in CARNs [32], resulted in tumor formation. Moreover, Germann and colleagues identified, in an androgen-dependent human xenograft, a castration-resistant, partially differentiated, luminal cell type, expressing ALDH1A1 and NANOG and the luminal markers NKX3-1 and CK18, and low levels of AR [49]. These cells survived castration in a quiescent state and started proliferating and differentiating after androgen replacement, regenerating the tumor mass [49].

In contrast, several independent studies using various model systems support a basal/undifferentiated cell origin for prostate CSCs. Normal prostate stem cells are long-lived, facilitating the accumulation of genetic and epigenetic mutations over the lifetime of an individual, while there is less opportunity for mutations to accumulate in post mitotic luminal cells. In this scenario, mutated stem cells give rise to aberrantly differentiated luminal cells with the ability to proliferate. It is indeed possible that a continuous activation of the stem cells due to chronic inflammation signals [50] could favor epigenetic and genetic instability, first resulting in proliferative inflammatory atrophy (PIA), followed by PIN, and consecutively PCa.

From a histological point of view, metastases and high grade cancers often include rare cells expressing basal cell markers, such as high-molecular-weight cytokeratins [37]. Moreover, it has been proposed that the castrate-resistant state results from clonal expansion of androgen-independent cells that are present at a frequency of 1 per 10^5–10^6 androgen-responsive cells [51].

Confirmation of a putative basal cancer stem cell came from our laboratory: where cells selected from human PCa biopsies with a $CD44^+/\alpha_2\beta_1 integrin^{hi}/CD133^+$ phenotype were able to self-renew in vitro and differentiate to an $AR^+/PAP^+/CK18^+$ luminal phenotype [52]. Findings from other laboratories support this idea: the $CD44^+$ population from tumor xenografts and cell lines (AR^-, OCT-4^+, and BMI-1^+) has enhanced proliferative potential and tumor-initiating ability in vivo compared to $CD44^-$ cells [53]. The side population, determined using Hoechst 33342 dye efflux

and selected from primary PCa tissues, exhibits a basal phenotype and has sphere-forming features [54]. Goldstein et al. (2010) reported that basal epithelial cells (but not luminal) from both mouse and human prostate were able to initiate tumors in immunodeficient mice when infected with AKT, ERG, and AR overexpressing vector and recombined with fetal urogenital sinus mesenchyme [55, 56]. In a conditional PTEN knockout mouse model for prostate cancer, an expansion of a basal stem/progenitor cell phenotype was observed after induction of PTEN deletion, with consequent tumor initiation [57]. More recently, it was shown that basal $Lin^{-}/Sca\text{-}1^{high}/CD49f^{high}$ cells have the capacity to form tumor-like spheroids in vitro and are tumorigenic in vivo [58]. In this model, the stromal component (cancer-associated fibroblasts) also played a crucial role in modulating the CSCs and stimulating tumor formation. Moreover, $TRA\text{-}1\text{-}60^{+}/CD151^{+}/CD166^{+}$ cells, isolated from human prostate xenografts, expressed basal cell markers and exhibited stem-like cell characteristics, recapitulating the cellular hierarchy of the original tumor in serial transplantation experiments [59]. The latter phenotype is shared with stem cells selected on the basis of CD133 expression from primary human tissues [52, 60].

An easy argument to resolve this discrepancy between basal and luminal stem cell phenotype could be that different cell types can exhibit CSC properties (e.g. regenerative potential and fate) depending on the environment (stem cell niche), the model used, and the type of experiment conducted. In agreement with this hypothesis is a recent paper from the Blanpain group on mouse breast stem cells, showing two separate lineages for myoepithelial and luminal cells in intact mammary tissue [61]. However, basal/myoepithelial stem cells were able to regenerate both basal and luminal cells, showing a greater potency and plasticity than luminal cells.

These observations are in accordance with a hierarchical model where a proportion of the regeneration potential is maintained in partially differentiated progenitor cells. Under physiological conditions, these progenitor cells (transit amplifying) are able to proliferate and differentiate to generate luminal cells, maintaining the epithelial turnover, while the stem cells remain in a quiescent state and do not need to be activated. The undifferentiated basal stem cells however seem to have more potential and are able to regenerate complete prostatic structures from even a single cell [31]. This hypothesis could explain also the current discrepancy observed in the prostate CSC field. It is possible that the genomic and phenotypic aberrancies accumulate in cancer progenitor cells conferring them with partial stem cell properties, such as self-renewal and regeneration, while the stem cells remain dormant and potentially reactivate only in response to treatment [62].

11.3 Prominin-1 Expression in the Prostate

The pentaspan membrane glycoprotein prominin-1 (CD133) is encoded (in humans) by the gene *PROM1* located on chromosome 4 [63]. Antibodies directed against the glycosylated form of this protein (AC133) have been used to select cells with stem cell proprieties from numerous tissues and tumors (reviewed in [64]). Using this

marker, Richardson and colleagues were able to enrich for cells with stem cell characteristics from the $\alpha_2\beta_1$integrin^{hi} fraction of basal prostate epithelial cells derived from primary tissues [27]. These cells possessed a high in vitro proliferative potential and were able to reconstitute prostatic acini in immunocompromised mice. Since this key publication, numerous other groups have used CD133 to select cells with stem cell features from many prostate model systems.

11.3.1 CD133 Expression in Human and Mouse Prostate Tissues

In 2004, Richardson and colleagues showed for the first time that a rare subpopulation of CD133$^+$ cells was present in the basal layer of the human prostate [27]. These cells are randomly scattered throughout the acinus and are either found alone or clustered at budding regions or branching points. More recently, Missol-Kolka and colleagues analyzed CD133 expression in the mouse prostate, finding it widely expressed on the luminal side of the epithelium [65]. This apparent inconsistency poses critical questions on the biology of CD133 in mouse and human prostate and on the use of antibodies specific for different protein epitopes. It is important to remember that the most used antibodies for marking and selecting stem cells from many different human adult tissues are directed against the AC133 or the 293C3/AC141 epitopes of the CD133 molecule [64]. These epitopes are located in a glycosylated portion of CD133 in the second extracellular loop [66] and seem to be particularly dependent on protein folding and glycosylation [67–69]. Other human-specific antibodies against the CD133 polypeptide have been developed, but these show a less restricted expression of CD133 [70, 71], suggesting that the corrected glycosylation and protein folding of CD133 is necessary for a precise marking of the stem cells, while throughout the body, CD133 seems to be expressed in many cell types in addition to the adult stem cells. To clarify this discrepancy, the Corbeil group compared the expression of CD133 in the human prostate using two different antibodies: AC133 and 80B258 (against the polypeptide chain) [65]. Interestingly, they revealed that in the basal layer of the prostate epithelium, only a small subpopulation of cells was marked by the 80B258 antibody and that this population seemed to coincide with the stem cell population stained by the AC133 antibody. However, the 80B258 antibody also showed positivity in a proportion of the luminal cells, typically with an apical membrane staining. These results mirrored the expression in the mouse prostate, clearly stating that CD133 expression in the prostate is indeed not restricted to the rare basal epithelial cells with stem cell features but that its expression is reacquired by terminally differentiated luminal cells but with a different conformation/glycosylation pattern. Understanding precisely how CD133 expression and posttranslational modifications are regulated throughout the prostate epithelial hierarchy (discussed in Sect. 11.4) and, more generally, how the AC133 epitope is regulated/masked are still unanswered questions in this field.

11.3.2 Histological Expression of CD133 in Prostate Cancer Tissues

In 2005, our laboratory published the first identification of stem-like cells in prostate cancer [52]. We reported that $CD44^{+}/\alpha_2\beta_1 integrin^{hi}/CD133^{+}$ cells from primary prostate cancers had the ability to self-renew and differentiate in vitro. Although the final confirmation that these cells have tumor-initiating capacity in vivo was not presented at the time, this publication indeed generated a wide interest in the role of CD133 expression in prostate cancer. Several publications showed that CD133 (AC133) positive cells exist within prostate cancer [54, 72, 73]; however, the percentage of positive cells varied considerably between publications. Eaton and colleagues showed that CD133 was expressed (at low frequency, <1%) in half of the primary cancers tested, and its expression was increased in matched metastasis [73]. Miki and colleagues reported that $CD133^{+}$ cells within prostate cancer tissues lacked nuclear AR expression, suggesting an undifferentiated phenotype [72]. In contrast with these reports, Missol-Kolka and colleagues reported no CD133 expression in 18 prostate cancer samples. This was a surprising result as, in this study, they used antibodies against the CD133 polypeptide chain, which has already been shown to be widely expressed in normal tissues [65].

11.4 Regulation of CD133 Expression in the Prostate

The histological expression pattern of CD133 in the normal prostate suggests that the gene is expressed specifically in basal stem cells (AC133 epitope) and, in a different isoform, in terminally differentiated luminal cells, while the vast majority of the basal and intermediate cells remain CD133 negative. This implies a very dynamic and tight control for CD133 expression throughout the prostate epithelial hierarchy (Fig. 11.1).

CD133 expression can be controlled at multiple steps including transcriptional regulation, alternative transcription initiation sites, alternative splicing, and post-translational modifications [74, 75].

In the prostate, transcriptional regulation, together with posttranslational and conformational changes, seems to be essential for the correct expression pattern of CD133. Within basal prostate cells, CD133 mRNA is expressed at high levels only in rare AC133-positive cells [76, 77], while the majority of the cells do not express this gene. CD133 is then reexpressed in luminal cells but in a non-AC133 reactive form. This dynamic transcriptional regulation fits with a model of changes in the activation of transcription factors and chromatin remodeling around the five independent promoters which regulate CD133 expression in a tissue-specific manner, producing transcripts containing an alternative first exon [78].

In prostate, expression is initiated by promoter P1, generating a transcript that includes exon 1A [78]. We have confirmed this result in prostate epithelial cell lines

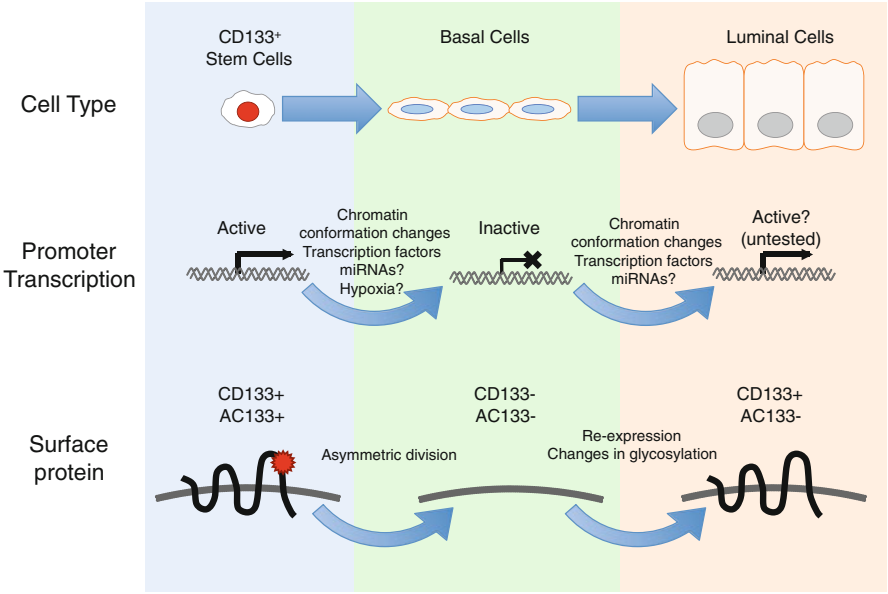

Fig. 11.1 Schematic representation of the proposed tight regulation of CD133 throughout the normal prostate epithelial hierarchy

(Fig. 11.2) by specific amplification of each alternative first exon by RT-PCR. The transcription factors which specifically regulate this promoter in prostate are still uncertain; however, insights can be drawn from studies conducted in other tissues. A strict relationship has been shown between hypoxia, hypoxia-inducible factor (HIF)1α and HIF2α transcription factors, and CD133 expression, although sometimes with opposite effects depending on the tissues studied [66, 79–81]. The overall consensus is however that hypoxic conditions stimulate CD133 expression and promote the expansion of CD133-expressing cells. This is accompanied by the upregulation of many other stem cell features in both prostate [82] and other tissues [80, 83–86]. Iida and colleagues showed that hypoxia induces CD133 expression in lung cancer cells. This induction is mediated by OCT4 and SOX2, both of which are induced by HIF1α and HIF2α, via their direct interaction with the P1 promoter [80]. Other studies reported the involvement of several other pathways and transcription factors in regulating CD133, such as the Ras/ERK pathway [87], the mTOR pathway [81], the TGF-β pathway (through DNA methylation) [88], and AF4 transcription factor [89]. However, CD133 transcriptional activation seems to be extremely tissue-specific, and more detailed studies on prostate and prostate cancer are still required.

Another widely reported mechanism, which regulates CD133 transcription, is the hypermethylation of its promoter. The CpG island present around promoters P1, P2, and P3 of CD133 is frequently hypermethylated in a number of normal tissues and tumors, inhibiting CD133 transcription [90–93]. Although the same mechanism is present in some prostate cell lines [76], this seems to be a result of culture adaptation

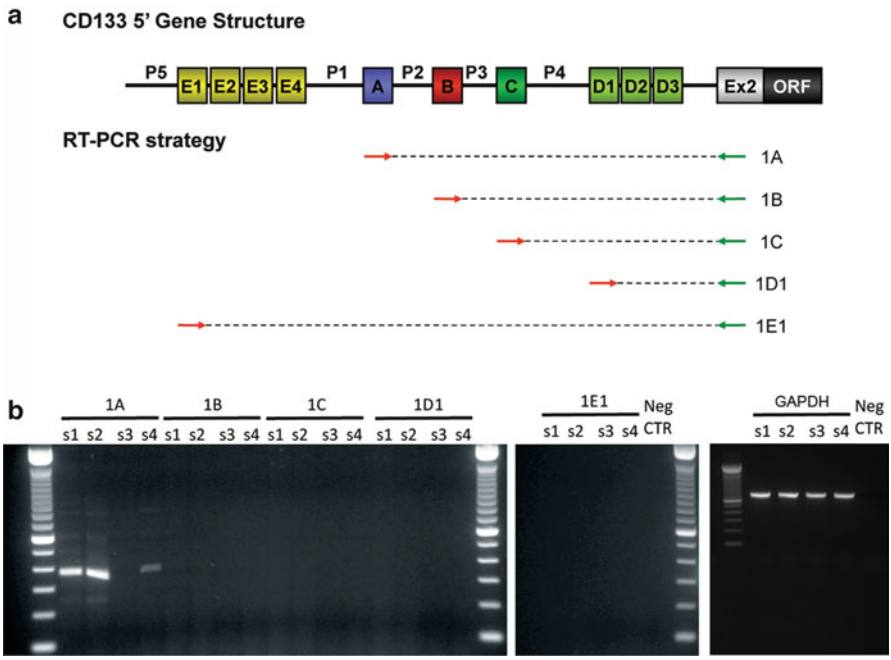

Fig. 11.2 (**a**) CD133 5' gene structure (*top panel*) (A,B,C,D1-3, E1-4 = alternative first exons; P1-P5 = promoters; Ex2 = Exon 2; ORF = open reading frame) and RT-PCR strategy for specific amplification of the alternative first exons (*bottom panel*). (**b**) Amplification of CD133 alternative first exons by RT-PCR in RC-165 N/hTERT and DU145 cell lines treated with 1 μM 5-Aza-2'-deoxycytidine for 96 h. s1 = RC-165 N/hTERT DMSO; s2 = RC-165 N/hTERT 5-Aza-2'-deoxycytidine; s3 = DU145 DMSO; s4 = DU145 5-Aza-2'-deoxycytidine

of these cells. In both prostate primary cultures and prostate tissues, CD133 regulation is independent of DNA methylation, and CD133 is almost always hypomethylated. The results in Fig. 11.3 clearly show that promoter-specific hypermethylation of CD133 to be the result of cellular adaptation to serial passaging (in this case, of a xenografted primary tumor) (Fig. 11.3) and that this process is not restricted to CD133. We have recently demonstrated that a more dynamic regulation involving chromatin condensation and a switch in histone marks seems to play the major role in regulating CD133 transcription in the prostate. The presence of active or inactive chromatin marks correlated perfectly with CD133 expression in prostate cell lines, while treatment with histone deacetylase inhibitors induced CD133 expression both in prostate cell lines and primary epithelial cultures with no involvement of DNA methylation [76]. Interestingly, our results also indicate that the tissue-specific choice of CD133 first exon is not under the control of DNA methylation in cell lines. Treatment of prostate cell lines with the demethylating agent 5-Aza-2'-deoxycytidine induced a marked reexpression of CD133 mRNA and AC133 protein [76]. Figure 11.2 shows that DU145 cells treated with 1 μM 5-Aza-2'-deoxycytidine for 96 h specifically reexpressed a CD133 isoform-containing exon 1A (Fig. 11.2),

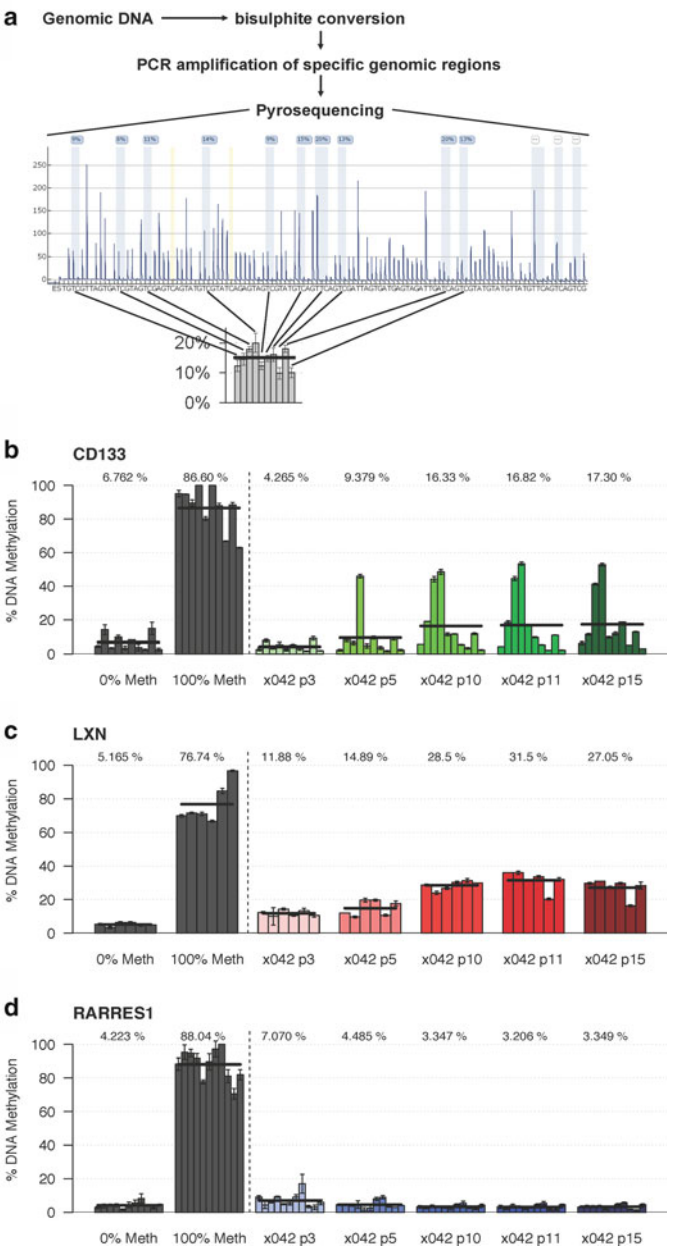

Fig. 11.3 Schematic representation of the pyrosequencing methylation analysis workflow (**a**). In brief: genomic DNA is bisulphite converted and the genomic region of interest is amplified by PCR; the PCR product is then sequenced by pyrosequencing [108] and a pyrogram is generated. DNA methylation percentages from each CpG site in the region are summarized in a bar plot with a horizontal line representing the average. Pyrosequencing methylation analysis of CD133 (**b**), LXN (**c**), and RARRES1 (**d**) performed in prostate primary xenografts throughout several passages spanning 1.5 years of culture (from passage 3 to passage 15) (bars = single CpG sites; $n = 3$ technical replicas; mean ± SD; line = average of all the CpG sites, 0% Meth = 0% methylation control, 100% Meth = 100% methylation control)

while the cell line RC-165 N/hTERT (which lack a hypermethylated *CD133* promoter) was used as a control.

The switch between an AC133-positive CD133 isoform in prostate basal stem cells and an AC133-negative isoform in luminal cells is indeed another riddle in prominin-1 biology. What is the difference between these two isoforms and how are they regulated? Yu and colleagues proposed that alternative splicing could regulate this. They showed that CD133-2 isoform, which lacks a small exon of 27 nucleotides, was expressed in hematopoietic stem cells and recognized by the anti-AC133 monoclonal antibodies [94]. On the other hand, Mak and colleagues showed that N-glycosylation processing was necessary for the correct recognition of the AC133 epitope, indicating that changes in glycosylation patterns or conformation alone could lead to a AC133-negative form of CD133 [69].

11.5 CD133 as a Stem Cell Marker in Prostate and Prostate Cancer

As discussed above, our laboratory was able to select cells with stem cell characteristics using the AC133 antibody from both benign and malignant prostate tissues [27, 52]. $\alpha_2\beta_1$integrin^{hi}/CD133$^+$ cells from benign prostatic tissue display a basal phenotype and are quiescent (Ki67$^-$), but have a much higher ability to form colonies and higher proliferative potential than $\alpha_2\beta_1$integrin^{hi}/CD133$^-$ cells. When implanted subcutaneously in immunocompromised mice, CD133$^+$ cells were able to form acini-like structure that recapitulated the entire spectrum of prostate differentiation.

In cancer tissues, CD44$^+$/$\alpha_2\beta_1$integrin^{hi}/CD133$^+$ cells had a very similar phenotype to their benign counterpart, displaying basal cell markers such as cytokeratin 5 and 14 [52]. These cells also showed a high colony forming ability and a proliferative potential much higher than their benign counterpart and were able to differentiate in vitro to luminal-like cancer cells. Indeed, cultures generated from these cells were more invasive in vitro compared to BPH cultures and displayed the frequent prostatic gene fusion TMPRSS2-ERG [36, 60]. Furthermore, $\alpha_2\beta_1$integrin^{hi}/CD133$^+$ prostate CSCs have a distinct gene expression profile relative to both their normal and differentiated counterpart [60], showing differential expression of genes associated with inflammation, cellular adhesion, and metastasis.

Since 2005, CD133 has been used by many other groups as a prostate (and PCa) stem cell marker in various models, sometimes however with inconsistent results. In hTERT immortalized prostate cell lines, AC133-positive cells displayed stem cell characteristics in vitro that mirrored cells from patient tissues [72]. However, another group showed that side population was a much better CSC marker in this model compared to CD133 [95].

Wei and colleagues also reported that CD44$^+$/$\alpha_2\beta_1$integrin^{hi}/CD133$^+$ cells are present in the DU145 cell line and possess cancer stem cell features in vitro and in vivo [96]. This result was then confirmed by Dubrovska, showing also that prostate cancer

cell lines grown in sphere-forming conditions increased the number of $CD44^+$/$CD133^+$ cells, in vitro and in vivo tumorigenic potential [97]. Moreover, the Shay group showed that $CD133^+$ cells from DU145 have higher telomerase activity [98]. However, others failed to see the same stem cell characteristic in $CD133^+$ DU145 cells [99]. This inconsistency in studies with the same, long established cell line may reflect heterogeneity and selection of dominant clones as seen in several other cellular models of cancer, rather than genuine changes in $CD133^+$ cell content [100].

Interestingly, Vander Griend and colleagues [101] demonstrated that a small subpopulation of $CD133^+$ cells was present in several cancer cell lines, which possessed a higher clonogenic potential. However, this subpopulation expressed AR, which is in stark contrast to all other reports showing that $CD133^+$ cells from human normal and cancerous tissues and cell lines have a basal phenotype [27, 52, 72, 77, 97, 102]. Moreover, many other groups failed to find a $CD133^+$ subpopulation in these cell lines [76, 99], confirming that CD133 can be highly dysregulated after long-term culture in vitro and the marker should be used with caution in cell lines.

The ultimate evidence for CSCs is reconstitution of a tumor in a recipient animal, which is identical to the parental tumor and that can be serially xenotransplanted indefinitely. Preliminary data from our laboratory showed that CD133+ cells selected form primary PCa xenografts were indeed able to form tumors from as few as 10 cells [36]. $\alpha_2\beta_1 integrin^{hi}/CD133^+$ cells selected from the BPH-1 cell line were also able to form tumors in mice when recombined with cancer-associated fibroblasts; however, they had a much lower tumor-initiating potential compared to $\alpha_2\beta_1 integrin^{hi}/CD133^-$ cells [103]. Serial transplantation (not performed in the latter study) is important to distinguish between bona fide stem cells and highly proliferating progenitor cells. Indeed, more work needs to be carried out in order to better assess the potential of CD133 as a bona fide stem cell marker in human prostate cancer; that is, identify whether it is a permanent marker or (more likely) one whose expression is condition and context dependent.

11.6 Conclusions

As discussed above, human prostate epithelial stem cells are quiescent and reside in the basal layer. Many groups were able to separate cells with prostate-regenerating capabilities from this compartment using several different markers. Indeed, basal cells expressing CD133 are quiescent stem cells able to regenerate differentiated luminal cells in vitro and in vivo [27, 28], confirming the validity of CD133 as a prostate stem cell marker.

However, the prostate cancer field is still debating on the nature of CSCs. In human prostate cancer, a solid body of evidence indicates that cells with a basal or partially differentiated (intermediate) phenotype have cancer stem cell characteristics and that their normal counterpart can function as a cell-of-origin for prostate cancer. In this scenario, the use of CD133 as a cancer stem cell marker is still somewhat uncertain: although several reports show that $CD133^+$ cells from various models

have cancer stem cell features, other groups were able to isolate prostate CSCs without the use of CD133 but with basal markers, such as CD44, ALDH, and $\alpha_2\beta_1$ integrin. This could be partially explained by the tight regulation needed for the correct expression of CD133, which is indeed influenced by niche and environmental conditions (especially after long-term culture in vitro and serial passaging in vivo), resulting in unstable expression of CD133 which is expressed only in the appropriate microenvironment. It is important to remember though that CD133 expression remains at the moment only a stem cell marker and not a stem cell feature, as sometimes reported in the literature. CD133 function is largely unknown, especially in the prostate, where it is expressed as two distinct isoforms in basal and luminal cells. CD133 is localized to plasma membrane protrusions where it interacts with membrane cholesterol [104]. These membrane microdomains could be enriched in components involved in maintaining stem cell properties, and their loss, perhaps through asymmetric cell division, might promote cell differentiation [105]. Interestingly, CD133 has been shown to segregate with the template DNA in asymmetric cell division in lung cancer cells, while differentiation markers were expressed only in the daughter cell [106]. Interestingly, the frequency of asymmetric cell division was enhanced by environmental factors such as cell-cell contact, serum, and hypoxia, all of which seem to play an important role in CD133 regulation. Moreover, a recent report showed that CD133 is a suppressor of differentiation in neuroblastoma [107], giving a first insight into CD133 function and linking this marker functionally to the maintenance of an undifferentiated (stem) phenotype.

It is likely that the definition of CD133 function and regulation in relation to prostate stem cells and CSCs will be of primary importance and should provide novel insights into the nature of the tumor initiation process.

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