Original Article
CD133, Trop-2 and α2β1 integrin surface receptors as markers of putative human prostate cancer stem cells

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Abstract: Cancer stem cells (CSCs) play a key role in initiation and development of cancer and are attractive targets for therapy. The identification of CSC surface receptors to be used as therapeutic targets in vivo remains a difficult task. In this study, we assessed the expression pattern of three surface receptors: CD133, Trop-2 and α2β1 integrin in human prostate cancer in order to identify CSC-niches. CD133 was found to be expressed in small clusters of cells localized in focal areas of benign as well as malignant lesions, suggesting that this protein is a bona fide marker of the prostate stem/progenitor compartment. Trop-2 was localized in both basal and luminal layers of benign glands and was highly expressed in malignant lesions. Moreover, isolated cells in benign and malignant areas were found to co-express both CD133 and Trop-2. α2β1 integrin was expressed in the prostatic epithelium as well as in the surrounding stroma, limiting its utility as a marker of CSCs. In summary, we demonstrate that the combination of CD133 and Trop-2 is useful to mark putative CSC-containing compartments in human prostate.

Introduction

The origin of prostate cancer (PrCa) is still a matter of debate in the scientific community [1]. The three specific lineages (basal, luminal and neuroendocrine) of the normal prostatic epithelium [2] result from the directional differentiation of normal stem cells (NSCs) [3]. The vast majority of PrCa cells has a luminal phenotype, but unlike normal luminal cells that are terminally differentiated and have limited proliferative capacity, PrCa cells still retain the ability to proliferate, resembling "basal" characteristics [4]. To date, two major theories have been proposed to explain the initiation of PrCa [5]. First, PrCa may arise from niches of NSCs that are localized in the basal layer of prostate glands [2, 6]. Under physiological conditions, these low-proliferating NSCs can give rise to a second population of more rapidly cycling cells, known as transit amplifying cells [7], which differentiate into mature, secretory cells [2]. However, during tumorigenesis genes able to tightly control cell proliferation or DNA damage repair in NSCs can accumulate "transforming" mutations, resulting in the conversion to cancer stem cells (CSCs) that show growth advantages over their normal counterparts [8, 9]. A second theory postulates that PrCa can originate from normal cells in the luminal/transit amplifying layer, that re-acquire abilities for self-renewal, initiating the uncontrolled tumor growth [10].

CSCs, also designated cancer stem/progenitor cells [11], are defined as cells with the special ability to generate tumors when injected in animal hosts [12] through processes of self-renewal and differentiation into the multiple

lineages of cancer cells that comprise a tumor [13]. In the tumor, CSCs form a distinct niche which is resistant to conventional treatments, such as radiotherapy [14, 15], and can be responsible for relapse and metastatic dissemination of the disease. Novel therapies able to kill both differentiating/differentiated cancer cells and CSCs are absolutely needed in order to improve survival and quality of life of cancer patients and avoid the use of invasive approaches, such as radical prostatectomy for PrCa patients. Surface receptors expressed at high levels in cancer cells and CSCs would be ideal targets for treatments with specific antibodies or small-molecule inhibitors. Several candidate markers (such as CD24, CD44, CD133, CD166, Trop-1, and some integrins) have been tested to date, alone or in combination with each other, but profiles elaborated for CSCs are still weakly useful, since strongly dependent on the tumor histotype [16].

The transmembrane protein CD133, also known as Prominin-1, is one of the most important markers of hematopoietic [17] and neural stem cells [18], and it has also been used to identify prostate NSCs [19, 20]. In addition, epithelial cells from human prostate sorted in two populations, CD133+ and CD133-, show a gene expression signature of putative stem/progenitor cells, with similarities to the gene expression profile of hormone-refractory prostate cancer cells, and transit amplifying cells, respectively [21]. CD133 has been successfully used for the identification of CSC niches in glioblastoma [22], colon cancer [23] and other solid carcinomas [24-26], suggesting a strong relevance of this protein for the activity of CSCs. On the other hand, both CD133+ and CD133- populations isolated from metastatic colon cancers [27] and lung adenocarcinoma cell lines [28] were recently shown to have comparable abilities to generate tumors when implanted into immunodeficient mice. Thus, the role of CD133 in CSCs still remains controversial [29].

The stem compartment is localized in the basal layer of epithelial tissues, and the interaction with the basement membrane appears critical for the maintenance of stem-activity [30, 31]. In cancer, this type of interaction can stimulate the necessary early steps of the metastatic cascade [32]. A fundamental role in the activity of stem cells can be hypothesized for integrin receptors, which are critical regulators of cell-extracellular matrix (ECM) interactions [33, 34]. A conditional knock-out of β1 integrins from basal cells of the mammary gland was demonstrated to severely affect the normal development of the mammary epithelium [35]. In addition, ablation of β1 in a transgenic mouse model of breast cancer resulted in dramatically impaired initiation and maintenance of mammary tumor growth in vivo [36], suggesting that β1 play a critical role in the very early steps of tumorigenesis. Recently, α2β1 integrin was proposed as a marker of stem compartments in the normal prostate [37]. Moreover, Keratinocyte Growth Factor was found to induce terminal differentiation of the α2β1^{hi} transit amplifying cells, with concomitant suppression of α2β1 [38]. This evidence suggests that β1 integrins (and in particular α2β1 in the prostate) can be essential for the maintenance of a functional stem niche. A CD44+/α2β1^{hi}/CD133+ population isolated from PrCa patients was shown to have enriched tumorigenic properties in vitro [39]. However, these cells were not investigated for their ability to reproduce the entire tumor in vivo, partially supporting the use of the α2β1^{hi}/CD133+ profile as a marker of the PrCa stem niche. The standard form of CD44 (CD44s) was reported to be localized in benign epithelium, mostly in basal cells but also somewhat in secretory cells, and its expression was undetectable in cancer except for the neuroendocrine cells [40]. In addition, CD44s was also found in lymphocytes and nerves surrounding the prostatic epithelium, raising doubts on its utility for the targeting of stem niches in vivo [40]. However, a splice variant isoform, CD44v7-10, emerges in cancer [41, 42] and is currently under investigation as a more specific PrCa stem cell marker.

The transmembrane protein Trop-2, a homologue of Trop-1/EpCAM which is expressed in tissues of epithelial origin and has an important role in the early stages of tumor progression [43, 44], is frequently up-regulated in many human carcinomas as compared with their normal counterparts, suggesting an involvement of this protein in tumor growth [45, 46]. CSCs have been demonstrated to re-express several embryonic genes such as *OCT4, NANOG, SOX2* [47]; thus, regulatory programs related to early embryonic development can be re-activated during cancer initiation. This evidence suggests a potential role of Trop-2, which is expressed at high levels during early embryonic development

in the trophoblast [43], in CSCs activity. Recently, Trop-2 was proposed as a novel marker for the identification of stem cells in murine normal prostate [48]. Lin-Sca1+CD49f^{hi} normal murine prostate cells showed low or high stem-like activity in vitro and in vivo, based on low or high expression levels of Trop-2, respectively [48]. However, Trop-2 expression has never been studied in human prostate.

In this study, we have investigated the expression pattern of CD133, α2β1 integrin and Trop-2 in human PrCa specimens by immunohistochemistry (IHC) in order to identify niches of cells with putative CSC-like activity. Our findings demonstrate that the combination of CD133 and Trop-2 is useful to mark putative CSC-containing compartments in human prostate.

Materials and methods

PrCa specimens

Tissue specimens from radical prostatectomies were provided by: the Cooperative Human Tissue Network (CHTN; other investigators may have received samples from these same tissues) and the Department of Pathology, University of Massachusetts Medical School. The samples were unidentifiable, discarded human tissues processed according to institution-approved protocols. Specimens were processed for IHC as described below.

Antibodies

Mouse monoclonal antibodies (Ab) to p63 (clone 4A4) and α2 integrin (clone HAS-3) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Goat polyclonal Ab to Trop-2 was purchased from R&D Systems. Rabbit polyclonal Ab to CD133 was purchased from Abcam. Mouse IgG (Vector Labs, Burlingame, CA), rabbit IgG (Vector Labs) and goat IgG (Santa Cruz Biotechnology) were used as negative controls.

CD133 immunohistochemical staining

Tissue specimens were processed as described [49]. Briefly, 4 μm sections were cut from formalin-fixed, paraffin-embedded specimens. Sections were deparaffinized by three changes in xylene for 10 minutes each, and then re-hydrated in an ethanol series of 100%, 95%, 70%, 50%, ddH$_2$O for 2 minutes each. Removal of endogenous peroxidase activity was performed by incubation in 3% hydrogen peroxide for 5 minutes. Antigen retrieval was performed by microwave for 8 minutes in a buffer containing 1 M urea, pH 8. Similar results were also obtained by steam heat in buffer containing 10 mM sodium citrate, pH 6. Blocking was performed by incubation in PBS supplemented with 5% normal goat serum and 1% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO) at room temperature (RT) for 30 minutes. Tissue sections were incubated with the Ab to CD133 diluted in PBS+0.5% BSA at 4°C, overnight. After three washes with PBS+0.05% Tween-20, sections were incubated with an alkaline phosphatase-conjugated goat anti-rabbit (AP-GAR, Invitrogen, Carlsbad, CA) at RT for 1 hour. Signal from AP-GAR was visualized by Fast-Red staining (PicTure Staining Kit, Invitrogen) following manufacturer's instructions. Finally, the sections were counterstained with Mayer's hematoxylin and mounted using an acqueous-based mounting medium (Clearmount, Invitrogen). Sections were examined on an Olympus BX41 microscope equipped with an Olympus DP12 camera.

Trop-2 immunohistochemical staining

Formalin-fixed, paraffin-embedded tissue specimens were processed as described above, with the following modifications. Blocking solution was PBS supplemented with 5% normal rabbit serum and 1% BSA. Sections were incubated with the Ab to Trop-2 at RT for 1 hour. After three washes, incubation with a biotinylated rabbit anti-goat IgG (bio-RAG, Vector Labs) was performed at RT for 1 hour. Streptavidin conjugated to horseradish peroxidase (HRP-streptavidin, Vector Labs) was then added to sections and incubation was performed at RT for 30 minutes. The immunoperoxidase reaction was visualized using the DAB Substrate-Chromogen System (Zymed®, Invitrogen) following the manufacturer's instructions. Finally, the sections were counterstained with Mayer's hematoxylin, de-hydrated in an ethanol series of 50%, 70%, 95%, 100%, 2 minutes each, cleared by three changes in xylene, 1 minute each, and mounted for visualization using a synthetic, toluene-based resin (Permount, Fisher Scientific, Pittsburgh, PA).

Trop-2/CD133 double immunohistochemical staining

Double staining on formalin-fixed, paraffin-embedded tissue specimens was performed using the PicTure Double Staining Kit (Invitrogen), following the manufacturer's instructions with some modifications. Briefly, blocking was performed by incubation with PBS+5%BSA at RT for 45 minutes. Incubation with the Ab to Trop-2 was performed at RT for 1 hour, followed by incubation with the Ab to CD133 at 4°C overnight. Sections were incubated with a HRP-conjugated donkey anti-goat (HRP-DAG) at RT for 1 hour. After five washes with PBS+0.05% Tween-20, sections were incubated with an AP-GAR at RT for 1 hour. The signal from Trop-2 was detected by HRP staining (brown color), and the signal from CD133 was detected by Fast-Red staining (red color). Stained tissue sections were examined as described before. Goat IgG and rabbit IgG were used as negative controls.

α2β1 integrin immunohistochemical staining

IHC staining was performed on 5μm sections prepared from snap-frozen prostatic tumors. Sections were fixed in acetone at -20°C, 10 minutes. Blocking was performed by incubation with PBS supplemented with 0.5% casein and 0.05% thimerosal at RT for 15 minutes. Incubation with the Ab to α2 integrin was performed at 4°C overnight. After three washes in PBS, sections were incubated with a biotinylated horse anti-mouse IgG (bio-HAM, Vector Labs) at RT for 1 hour. HRP-streptavidin was used for signal amplification and signal was developed using the DAB Substrate-Chromogen System. Finally, the sections were mounted for visualization using the Clearmount medium.

p63 immunohistochemical staining

Tissue samples were processed as described above, with the following modifications. Antigen retrieval was performed by microwave for 15 minutes in a buffer containing 10 mM sodium citrate, pH 6. Blocking solution was PBS supplemented with 50% normal horse serum and 1% BSA. Sections were incubated with the Ab to p63 at 4°C overnight. Then, incubation with a bio-HAM was performed at RT for 1 hour.

Results

In this study, we performed a semi-quantitative immunohistochemical analysis of expression and localization of three surface receptors: CD133, Trop-2 and α2 integrin (which is known to form complexes with β1 integrin subunit, and is from here designated as α2β1) in human PrCa specimens in order to identify niches containing epithelial cells with putative CSC-like activity.

In nine out of ten specimens from patients with localized PrCa, CD133 was found to be expressed in isolated cells or small clusters in both benign glands and malignant lesions (**Figure 1**), with a pattern expected for niches containing putative CSCs.

Then, we investigated the expression of Trop-2 in sixteen human PrCa specimens. The role of Trop-2 has been previously demonstrated in murine normal prostate development [48], but
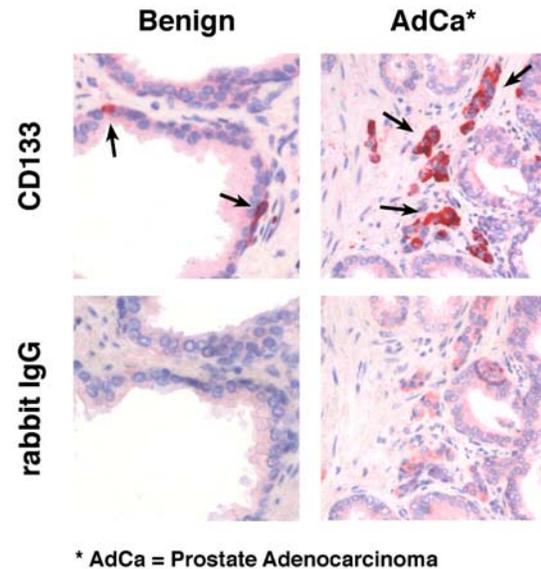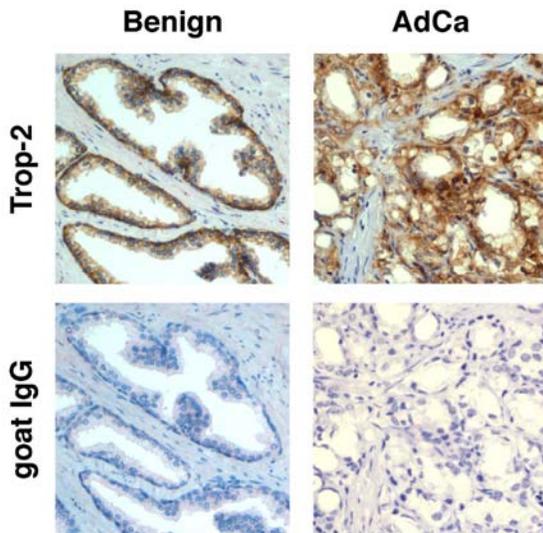

Figure 1. CD133 expression in benign and malignant prostate. CD133 immunostaining was performed using formalin-fixed, paraffin-embedded human PrCa sections. CD133 was detected (arrows) in isolated cells localized in the basal layer of benign prostate glands (left) or in small clusters in malignant areas (Gleason grade 4; right). Rabbit IgG, negative control.

its expression has never been investigated in human prostate. In all the cases analyzed here, Trop-2 was found to be localized in epithelial cells of benign and malignant areas with undetectable expression in the surrounding stroma (**Figure 2**). Its expression levels in benign prostate were higher in the basal layer as compared

with the luminal layer and were significantly increased in PrCa (**Figure 2**).

Analysis performed by double-staining IHC detected cells co-expressing both Trop-2 and CD133 at high levels in benign and malignant lesions (**Figure 3A**, arrows). As shown in **Figure 3B** (high magnification box), cells co-expressing both Trop-2 and CD133 (double staining, arrow)

Figure 2. Trop-2 expression in PrCa. Trop-2 immunostaining was performed using formalin-fixed, paraffin-embedded human PrCa sections. Trop-2 was found to be expressed at high levels in both benign (left) and malignant (Gleason grade 4; right) epithelial cells. Goat IgG, negative control.

were clearly distinguishable from cells stained for CD133 or Trop-2 (single staining, arrowheads), suggesting that the use of these markers may help the identification of putative CSC niches in the prostatic epithelium.

We also analyzed the expression pattern of α2β1 integrin in six human PrCa specimens. α2β1 integrin has been proposed to play an important role for human prostate NSCs in vitro [37] even though its role in vivo is still unclear. In all the cases analyzed, α2β1 was found to be expressed in the prostatic epithelium (**Figure 4**, arrows) as well as in the surrounding stroma (**Figure 4**, arrowheads). Moreover, α2β1 was expressed at comparable levels in both basal and luminal layers of the glands. Thus, this pattern raises doubts on the use of α2β1 as a marker for isolation of CSCs in human PrCa
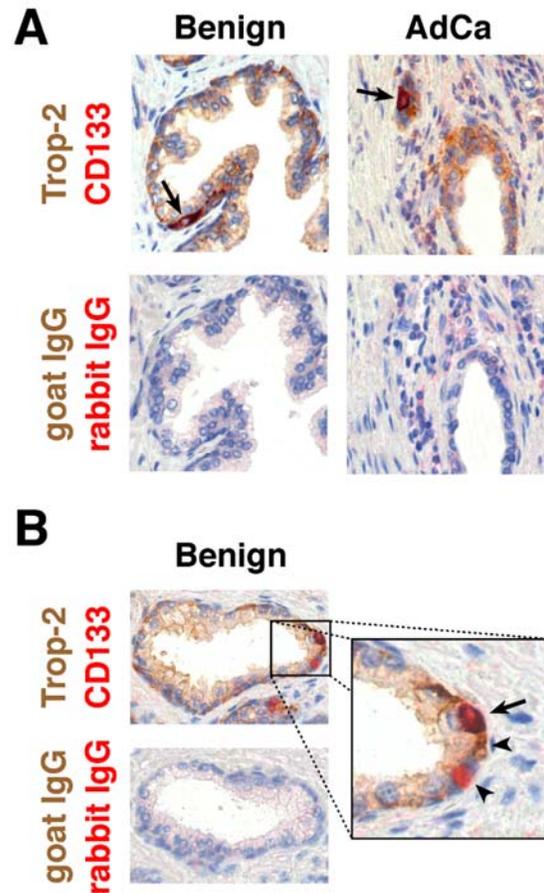
Figure 3. Co-expression of Trop-2 and CD133 in putative CSC cells in PrCa. (A) Co-staining of Trop-2 and CD133 in benign (left) and malignant (right) areas. Double staining appears as dark red (arrows). Rabbit IgG and goat IgG, negative controls. (B) A benign gland is shown in the low-magnification panel, and a detail is shown in the high-magnification box. A cell co-expressing both Trop-2 and CD133 (arrow) appears distinguishable from cells expressing only CD133 or Trop-2 (arrowheads). Rabbit IgG and goat IgG, negative controls.

specimens.

Six specimens from patients with PrCa were also analyzed for expression of p63. This protein is a homologue of the tumor suppressor protein p53, and was previously reported to be expressed in the basal layer of benign prostate glands and down-regulated in PrCa [50, 51]. In all the cases analyzed, we found strong nuclear localization of p63 in basal epithelial cells of the benign glands (**Figure 5A**, and arrow in **Figure 5B**), whereas in malignant areas its expression
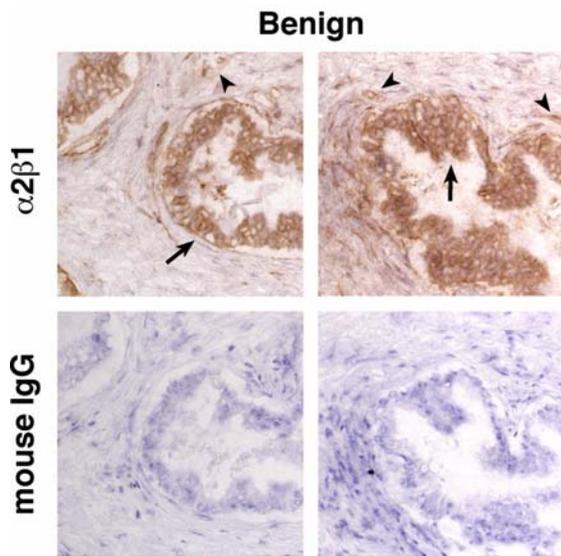
Figure 4. α2β1 integrin expression in PrCa. α2β1 integrin immunostaining was performed using sections from snap-frozen prostatic tumors. Expression of this protein was detected in epithelial cells of benign glands (arrows), and in the surrounding stroma (arrowheads). Mouse IgG, negative control.

Figure 5. p63 expression in benign and malignant prostate. p63 immunostaining was performed using formalin-fixed, paraffin-embedded PrCa sections to discriminate between benign and malignant areas. p63 showed nuclear localization in the basal cells of benign glands (A). p63 was undetectable in malignant lesions (Gleason grade 4; B) and selectively found in the nearby benign area (B, arrow). Mouse IgG, negative control.
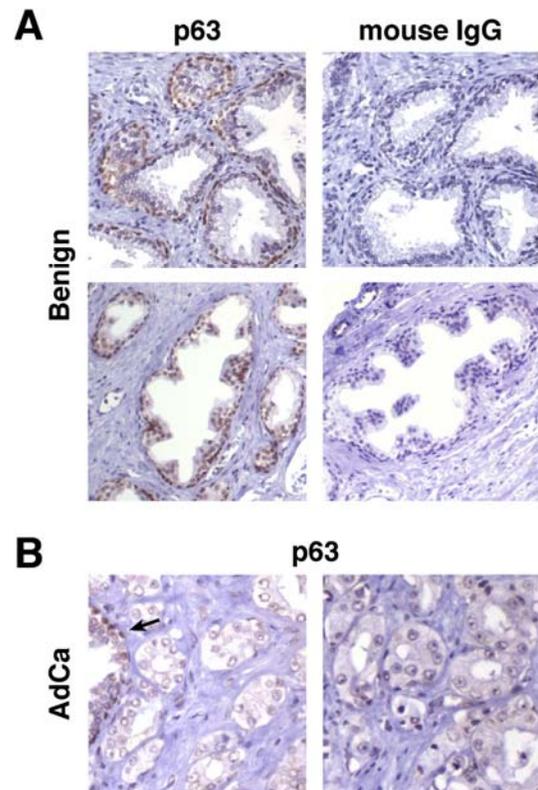
was undetectable (**Figure 5B**). These findings support p63 role as a marker to be used uniquely for the differential diagnosis of human PrCa by IHC.

Altogether, the present analysis suggests the combined use of CD133 and Trop-2 surface receptors for the isolation and characterization of putative CSCs in human prostate.

Discussion

In this study, we demonstrate for the first time that in human PrCa clusters of cells with putative CSC-like activity co-express CD133 and Trop-2. This novel markers' combination is proposed for prospective identification of putative CSCs in human prostate. At variance with previous reports [37], the wide expression pattern of α2β1 in both basal and luminal prostatic epithelium as well as in the surrounding stroma does not support the use of this protein, alone or in combination with other markers, for identification of CSCs in human prostate.

CSCs represent ideal targets for design of novel anti-cancer drugs, particularly in those cases where conventional treatments, such as radiation therapy [14, 15], are ineffective. Therefore, novel therapies able to target both cancer cells and CSCs are needed for prostate as well as for other types of cancer. Although proteins with selective expression in normal versus cancer (such as p63) were already proposed for diagnosis of PrCa [51] and for identification of CSCs [50], in this study we sought to analyze markers expressed on the cell surface as potential targets for therapeutic treatments, like antibody-mediated therapies. Given the co-expression of CD133 and Trop-2 in clusters of putative CSCs, this novel combination of surface receptors could be used to develop specific compounds for targeted therapy.

Trop-2 appears to be significantly up-regulated in PrCa as compared with benign luminal cells, which are known to give rise to PrCa. Since Trop-2 is found to be expressed in the basal layer of benign glands, it may not be excluded that the CD133$^+$/Trop-2$^+$ profile can identify prostatic NSCs. In this event, we speculate that cancer-specific aberrations of post-translational modifications may allow discrimination between NSCs and CSCs. Cancer-related modifications of N-linked glycosylation patterns are not infrequent, as seen for several proteins [52, 53], including Prostate Specific Antigen (PSA) and the heat-shock protein gp96 in PrCa [54-57]. Moreover, altered N-glycosylation of β1 integrins was also reported to deregulate the invasive potential of human fibrosarcoma and colon carcinoma cells [58, 59]. In support of this hypothesis, CD133 was recently demonstrated to undergo differential glycosylation in colon CSCs as compared with differentiated cancer cells [60]. This phenomenon could explain some discordant observations that have been reported when the CD133 protein and mRNA expression patterns were compared [61]. As recently proposed by Kemper *et al.*, differential glycosylation of the extracellular domain of CD133 can shield the epitope from detection by IHC using the antibody AC133 [60]. However, in the present study an antibody to the C-terminal, intracellular domain of CD133 was used, avoiding potential masking of the differentially glycosylated variants. Trop-2 has been found to be differentially glycosylated in transformed vs normal keratinocytes [62], suggesting potential post-translational mechanisms of regulation of this molecule in cancer. Thus, aberrant glycosylation of CD133 or Trop-2 in prostate CSCs may affect their folding and stability, resulting in incorrect protein assembly and abnormal signaling networks, thus promoting cancer growth.

Acknowledgments

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References

[1] Maitland NJ and Collins AT. Prostate cancer stem cells: a new target for therapy. J. Clin. Oncol. 2008; 26: 2862-2670.

[2] De Marzo AM, Nelson WG, Meeker AK and Coffey DS. Stem cell features of benign and malignant prostate epithelial cells. J. Urol. 1998; 160: 2381-2392.

[3] Robinson EJ, Neal DE and Collins AT. Basal cells are progenitors of luminal cells in primary cultures of differentiating human prostatic epithelium. Prostate 1998; 37: 149-160.

[4] Kasper S. Exploring the origins of the normal prostate and prostate cancer stem cell. Stem Cell Rev. 2008; 4: 193-201.

[5] Nikitin AY, Matoso A and Roy-Burman P. Prostate stem cells and cancer. Histol. Histopathol. 2007; 22: 1043-1049.

[6] De Marzo AM, Meeker AK, Epstein JI and Coffey DS. Prostate Stem Cell Compartments: Expression of p27^{kip1} in normal, hyperplastic and neoplastic cells. Am. J. Pathol. 1998; 153: 911-919.

[7] Isaacs JT and Coffey DS. Etiology and disease process of benign prostatic hyperplasia. Prostate Suppl. 1989; 2: 33-50.

[8] Lobo NA, Shimono Y, Qian D and Clarke MF. The biology of cancer stem cells. Annu. Rev. Cell Dev. Biol. 2007; 23: 675-699.

[9] Reya T, Morrison SJ, Clarke MF and Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105-111.

[10] Lang SH, Frame FM and Collins AT. Prostate cancer stem cells. J. Pathol. 2009; 217: 299-306.

[11] Mimeault M, Mehta PP, Hauke R and Batra SK. Functions of normal and malignant prostatic stem/progenitor cells in tissue regeneration and cancer progression and novel targeting therapies. Endocr. Rev. 2008; 29: 234-252.

[12] Regenbrecht CR, Lehrach H and Adjaye J. Stem-

ming cancer: functional genomics of cancer stem cells in solid tumors. Stem Cell Rev. 2008; 4: 319-328.

[13] Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL and Wahl GM. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res. 2006; 66: 9339-9344.

[14] Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD and Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006; 444: 756-760.

[15] Baumann M, Krause M and Hill R. Exploring the role of cancer stem cells in radioresistance. Nat. Rev. Cancer 2008; 8: 545-554.

[16] Marhaba R, Klingbeil P, Nuebel T, Nazarenko I, Buechler MW and Zoeller M. CD44 and EpCAM: cancer-initiating cell markers. Curr. Mol. Med. 2008; 8: 784-804.

[17] Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J and Buck DW. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 1997; 90: 5002-5012.

[18] Lee A, Kessler JD, Read TA, Kaiser C, Corbeil D, Huttner WB, Johnson JE and Wechsler-Reya RJ. Isolation of neural stem cells from the postnatal cerebellum. Nat. Neurosci. 2005; 8: 723-729.

[19] Miki J, Furusato B, Li H, Gu Y, Takahashi H, Egawa S, Sesterhenn IA, McLeod DG, Srivastava S and Rhim JS. Identification of putative stem cell markers, CD133 and CXCR4, in hTERT-immortalized primary nonmalignant and malignant tumor-derived human prostate epithelial cell lines and in prostate cancer specimens. Cancer Res. 2007; 67: 3153-3161.

[20] Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ and Collins AT. CD133, a novel marker for human prostatic epithelial stem cells. J. Cell Sci. 2004; 117: 3539-3545.

[21] Shepherd CJ, Rizzo S, Ledaki I, Davies M, Brewer D, Attard G, de Bono J and Hudson DL. Expression profiling of CD133+ and CD133- epithelial cells from human prostate. Prostate 2008; 68: 1007-1024.

[22] Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD and Dirks PB. Identification of human brain tumour initiating cells. Nature 2004; 432: 396-401.

[23] O'Brien CA, Pollett A, Gallinger S and Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007; 445: 106-110.

[24] Curley MD, Therrien VA, Cummings CL, Sergent PA, Koulouris CR, Friel AM, Roberts DJ, Seiden MV, Scadden DT, Rueda BR and Foster R. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. Stem Cells 2009; 27: 2875-2883.

[25] Monzani E, Facchetti F, Galmozzi E, Corsini E, Benetti A, Cavazzin C, Gritti A, Piccinini A, Porro D, Santinami M, Invernici G, Parati E, Alessandri G and La Porta CA. Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. Eur. J. Cancer 2007; 43: 935-946.

[26] Suetsugu A, Nagaki M, Aoki H, Motohashi T, Kunisada T and Moriwaki H. Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. Biochem. Biophys. Res. Commun. 2006; 351: 820-824.

[27] Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T, St Clair R, Baljevic M, White I, Jin DK, Chadburn A, Murphy AJ, Valenzuela DM, Gale NW, Thurston G, Yancopoulos GD, D'Angelica M, Kemeny N, Lyden D and Rafii S. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. J. Clin. Invest. 2008; 118: 2111-2120.

[28] Meng X, Li M, Wang X, Wang Y and Ma D. Both CD133+ and CD133- subpopulations of A549 and H446 cells contain cancer-initiating cells. Cancer Sci. 2009; 100: 1040-1046.

[29] LaBarge MA and Bissell MJ. Is CD133 a marker of metastatic colon cancer stem cells? J. Clin. Invest. 2008; 118: 2021-2024.

[30] Raymond K, Deugnier MA, Faraldo MM and Glukhova MA. Adhesion within the stem cell niches. Curr. Opin. Cell Biol. 2009; 21: 623-629.

[31] Scadden DT. The stem-cell niche as an entity of action. Nature 2006; 441: 1075-1079.

[32] Klarmann GJ, Hurt EM, Mathews LA, Zhang X, Duhagon MA, Mistree T, Thomas SB and Farrar WL. Invasive prostate cancer cells are tumor initiating cells that have a stem cell-like genomic signature. Clin. Exp. Metastasis 2009; 26: 433-446.

[33] Goel HL, Alam N, Johnson IN and Languino LR. Integrin Signaling aberrations in Prostate Cancer. Am. J. Transl. Res. 2009; 1: 211-220.

[34] Goel HL, Li J, Kogan S and Languino LR. Integrins in prostate cancer progression. Endocr. Relat. Cancer 2008; 3: 657-664.

[35] Taddei I, Deugnier MA, Faraldo MM, Petit V, Bouvard D, Medina D, Fassler R, Thiery JP and Glukhova MA. β1 integrin deletion from the basal compartment of the mammary epithelium affects stem cells. Nat. Cell Biol. 2008; 10: 716-722.

[36] White DE, Kurpios NA, Zuo D, Hassell JA, Blaess S, Mueller U and Muller WJ. Targeted disruption of β1-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. Cancer Cell 2004; 6: 159-170.

[37] Collins AT, Habib FK, Maitland NJ and Neal DE. Identification and isolation of human prostate epithelial stem cells based on α2β1-integrin expression. J. Cell Sci. 2001; 114: 3865-3872.

[38] Heer R, Collins AT, Robson CN, Shenton BK and

Leung HY. KGF suppresses α2β1 integrin function and promotes differentiation of the transient amplifying population in human prostatic epithelium. J. Cell Sci. 2006; 119: 1416-1424.

[39] Collins AT, Berry PA, Hyde C, Stower MJ and Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 2005; 65: 10946-10951.

[40] Palapattu GS, Wu C, Silvers CR, Martin HB, Williams K, Salamone L, Bushnell T, Huang LS, Yang Q and Huang J. Selective expression of CD44, a putative prostate cancer stem cell marker, in neuroendocrine tumor cells of human prostate cancer. Prostate 2009; 69: 787-798.

[41] Handorean AM, Yang K, Robbins EW, Flaig TW and Iczkowski KA. Silibinin suppresses CD44 expression in prostate cancer cells. Am. J. Transl. Res. 2009; 1: 80-86.

[42] Yang K, Handorean AM and Iczkowski KA. MicroRNAs 373 and 520c are downregulated in prostate cancer, suppress CD44 translation and enhance invasion of prostate cancer cells in vitro. Int. J. Clin. Exp. Pathol. 2009; 2: 361-369.

[43] Lipinski M, Parks DR, Rouse RV and Herzenberg LA. Human trophoblast cell-surface antigens defined by monoclonal antibodies. Proc. Natl. Acad. Sci. U.S.A. 1981; 78: 5147-5150.

[44] Zanna P, Trerotola M, Vacca G, Bonasera V, Palombo B, Guerra E, Rossi C, Lattanzio R, Piantelli M and Alberti S. Trop-1 are conserved growth stimulatory molecules that mark early stages of tumor progression. Cancer 2007; 110: 452-464.

[45] Fong D, Moser P, Krammel C, Gostner JM, Margreiter R, Mitterer M, Gastl G and Spizzo G. High expression of TROP2 correlates with poor prognosis in pancreatic cancer. Br. J. Cancer 2008; 99: 1290-1295.

[46] Ohmachi T, Tanaka F, Mimori K, Inoue H, Yanaga K and Mori M. Clinical significance of TROP2 expression in colorectal cancer. Clin. Cancer Res. 2006; 12: 3057-3063.

[47] Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A and Weinberg RA. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat. Genet. 2008; 40: 499-507.

[48] Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP and Witte ON. Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. Proc. Natl. Acad. Sci. U. S. A. 2008; 105: 20882-20887.

[49] Fornaro M, Tallini G, Zheng DQ, Flanagan WM, Manzotti M and Languino LR. p27(kip1) acts as a downstream effector of and is coexpressed with the β1C integrin in prostatic adenocarcinoma. J. Clin. Invest. 1999; 103: 321-329.

[50] Parsons JK, Saria EA, Nakayama M, Vessella RL, Sawyers CL, Isaacs WB, Faith DA, Bova GS, Samathanam CA, Mitchell R and De Marzo AM. Comprehensive mutational analysis and mRNA isoform quantification of TP63 in normal and neoplastic human prostate cells. Prostate 2009; 69: 559-569.

[51] Weinstein MH, Signoretti S and Loda M. Diagnostic utility of immunohistochemical staining for p63, a sensitive marker of prostatic basal cells. Mod. Pathol. 2002; 15: 1302-1308.

[52] Pinho SS, Osorio H, Nita-Lazar M, Gomes J, Lopes C, Gartner F and Reis CA. Role of E-cadherin N-glycosylation profile in a mammary tumor model. Biochem. Biophys. Res. Commun. 2009; 379: 1091-1096.

[53] Ueda K, Katagiri T, Shimada T, Irie S, Sato TA, Nakamura Y and Daigo Y. Comparative profiling of serum glycoproteome by sequential purification of glycoproteins and 2-nitrobenzensulfenyl (NBS) stable isotope labeling: a new approach for the novel biomarker discovery for cancer. J. Proteome Res. 2007; 6: 3475-3483.

[54] Kumar V, Hassan MI, Singh AK, Dey S, Singh TP and Yadav S. Strategy for sensitive and specific detection of molecular forms of PSA based on 2DE and kinetic analysis: a step towards diagnosis of prostate cancer. Clin. Chim. Acta. 2009; 403: 17-22.

[55] Peracaula R, Tabares G, Royle L, Harvey DJ, Dwek RA, Rudd PM and de Llorens R. Altered glycosylation pattern allows the distinction between prostate-specific antigen (PSA) from normal and tumor origins. Glycobiology 2003; 13: 457-470.

[56] Suriano R, Ghosh SK, Ashok BT, Mittelman A, Chen Y, Banerjee A and Tiwari RK. Differences in glycosylation patterns of heat shock protein, gp96: implications for prostate cancer prevention. Cancer Res. 2005; 65: 6466-6475.

[57] White KY, Rodemich L, Nyalwidhe JO, Comunale MA, Clements MA, Lance RS, Schellhammer PF, Mehta AS, Semmes OJ and Drake RR. Glycomic characterization of prostate-specific antigen and prostatic acid phosphatase in prostate cancer and benign disease seminal plasma fluids. J. Proteome Res. 2009; 8: 620-630.

[58] Guo HB, Lee I, Kamar M, Akiyama SK and Pierce M. Aberrant N-glycosylation of β1 integrin causes reduced α5β1 integrin clustering and stimulates cell migration. Cancer Res. 2002; 62: 6837-6845.

[59] Shaikh FM, Seales EC, Clem WC, Hennessy KM, Zhuo Y and Bellis SL. Tumor cell migration and invasion are regulated by expression of variant integrin glycoforms. Exp. Cell Res. 2008; 314: 2941-2950.

[60] Kemper K, Sprick MR, de Bree M, Scopelliti A, Vermeulen L, Hoek M, Zeilstra J, Pals ST, Mehmet H, Stassi G and Medema JP. The AC133 epitope, but not the CD133 protein, is lost upon cancer stem cell differentiation. Cancer Res. 2010; 70: 719-729.

[61] Bidlingmaier S, Zhu X and Liu B. The utility and limitations of glycosylated human CD133 epitopes in defining cancer stem cells. J. Mol. Med. 2008; 86: 1025-1032.

[62] Schon MP and Orfanos CE. Transformation of human keratinocytes is characterized by quantitative and qualitative alterations of the T-16 antigen (Trop-2, MOv-16). Int. J. Cancer 1995; 60: 88-92.