BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-17-039

Title: Cancer stem cell surface markers on normal stem cells

Article Type: Mini Review

Keywords: Cancer stem cells; surface marker; human embryonic stem cells; adult stem cells; normal tissue cells

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Cancer stem cell surface markers on normal stem cells

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Abstract

The cancer stem cell (CSC) hypothesis has captured the attention of many scientists because the elimination of CSCs is considered possible to eradicate the whole cancer. CSC surface markers provide molecular targeted therapies for the origins of cancer by using therapeutic antibodies. Various CSC surface markers have been identified and published. Interestingly, most of the markers used to identify CSCs are derived from the surface markers present on human embryonic stem cells (hESCs) or adult stem cells. In this review, we classify currently known 40 CSC surface markers into 3 different categories in terms of their expression in hESCs, adult stem cells, and normal tissue cells. Approximately 73% of current CSC surface markers appear to be present on embryonic or adult stem cells, and they are rarely expressed on normal tissue cells. The rest of the CSC surface markers are considerably expressed even in normal tissue cells, although some of them have been extensively validated as CSC surface markers by many research group. We discuss the significance of the categorized CSC surface markers and provide insight into why surface markers on hESCs will be an attractive source to find novel surface markers on CSCs.

Keywords: Cancer stem cells, surface marker, human embryonic stem cells, adult stem cells, normal tissue cells

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INTRODUCTION

Scientific knowledge about cancer formation and progression has explosively expanded over the past two decades. Cancers are regarded as aberrant and heterogeneous tissues containing a variety of cells that originate from a unique and rare subset of cancer cells with self-renewal capacity and potential to differentiate into multiple cell lineages (1). Rare subset of cancer cells with stem-like properties, referred to as cancer stem cells (CSCs) or tumor initiating cells (TICs), is responsible for cancer initiation, progression, and dissemination to distant organs (1, 2). The first prospective identification of CSCs was carried out with acute myeloid leukemia (AML), in which the surface markers of leukemic stem cells were defined as $CD34^{+}CD38^{-}$ phenotype (3). When transplanted into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, the small immature subset of $CD34^{+}CD38^{-}$ cells was able to reinitiate the same leukemia in NOD/SCID mice, while the major abundant subset of $CD34^{+}CD38^{+}$cells was not (3). The results showed for the first time the existence of CSCs in liquid tumor and promoted many researchers to use various cell surface markers to isolate CSCs from heterogeneous cell populations of solid tumor tissues. Since then, CSCs have been isolated from various solid tumors, such as breast (4), brain (5), prostate (6), pancreas (7), colon (8), lung (9), stomach (10), ovary (11), liver (12), and skin (13). After the identification of various CSCs, many researchers believe that the specific elimination of CSCs will lead to the disappearance of whole tumors based on the concept that the sole source of tumor self-renewal is CSCs. Since CSCs were identified on the basis of their cell surface molecules, specific antibodies/immunotoxins against the surface molecules were also successfully developed to eradicate CSCs selectively (14-17). Although there are still some doubts about the therapeutic strategies targeting CSCs, the approaches are expected to lead to better clinical outcomes in cancer patients by halting tumor progression (15, 18).

The development of therapeutic strategies targeting CSCs mainly relies on the use of cell surface markers to identify, enrich, and/or isolate CSCs. Many CSC surface markers have been identified, although some surface markers are controversial and need further investigation (1, 2, 19). Interestingly, most of the current CSC surface markers are derived from surface markers known to be present on normal embryonic or adult stem cells (1, 2, 19-21). The sharing of cell surface markers suggests that CSCs predominantly originate from

normal stem cells via the accumulation of epigenetic and genetic alteration (20). In this review, current 40 published CSC surface markers are classified into 3 different categories, depending on their expression on hESCs, adult stem cells, and normal tissue cells. The first group of CSC surface markers are expressed on hESCs, but they are weakly or rarely expressed on normal tissue cells (Table 1). The second group of CSC surface markers are expressed on adult stem cells, but they are weakly or rarely expressed on normal tissue cells (Table 2). The third group of CSC surface markers are expressed on hESCs and/or adult stem cells, but they are also considerably expressed on various normal tissue cells (Table 3). In the tables, histological data of some CSC surface markers originate from the human protein atlas (http://www.proteinatlas.org/), if they have not been published before. CD133 has been most frequently studied as a CSC surface marker in various cancer cells and specific antibodies/immunotoxins against CD133 were successfully developed to eradicate CSCs selectively (14, 17). CD133 expression is detected in 22 of 82 cell types from 44 normal human tissues (approximately 27%) (http://www.proteinatlas.org/). Based on the rate of CD133 expression, a CSC surface marker is classified as rare expression in normal tissue cells, if the marker is detected less than 27% (< 22 out of 82 normal tissue cells).

CSC surface markers expressed on hESCs, but rarely expressed in normal tissue cells

CSC surface markers that are expressed on hESCs, but rarely expressed in normal tissue cells, are summarized in Table 1. Stage-specific embryonic antigen 3 (SSEA-3) and SSEA-4 are epitopes on related glycosphingolipids. SSEA-3 and SSEA-4 play a key role in identifying hESCs (22). SSEA-3 is expressed on adult human mesenchymal stem cells (MSCs) (23) while SSEA-4 is expressed on mesenchymal and cardiac stem cells (24, 25). SSEA-3 and SSEA-4 have been demonstrated to express on breast cancer cells and breast CSCs (26). TRA-1-60 and TRA-1-81 antigens are expressed on podocalyxin in human pluripotent stem cells (hPSCs) (27). TRA-1-60 and TRA-1-81 are related to breast cancer (28). TRA-1-60 is also expressed on a minor subset of stem-like human prostate TICs (29). SSEA-1 is a cell surface marker for neural stem cells (NSCs) as well, functions in brain tumor stem cells, including self-renewal, multi-differentiation, and the ability to recapitulate the phenocopy of

primary tumors (30). SSEA-1 is also related to lung and renal tumors (31, 32). SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, and SSEA-1 are all carbohydrate epitopes and well-characterized oncofetal antigens, which are rarely expressed in adult normal differentiated tissues and cells. They are all hESC surface markers, except for SSEA-1.

CD133 (Prominin-1) is a glycosylated, 115-120-kDa protein with five transmembrane domains and two large extracellular loops (33). While the precise function of CD133 remains unknown, it has been proposed to act as an organizer of cell membrane topology (34). CD133 was initially discovered as a target of AC133 monoclonal antibody (MAb), specific for the $CD34^+$ population of hematopoietic stem cells (HSCs) (35). CD133 is expressed on the surface of hESCs (36) and NSCs (37) and downregulated upon the differentiation of hESCs, suggesting that CD133 expression is restricted to undifferentiated hESCs (36). The cell surface expression of CD133 antigen is among those that have been most frequently studied in solid cancers (33). CD133 has been used to define CSC populations in the breast, brain, lung, pancreas, liver, prostate, ovary, colon, and head and neck cancers, and $CD133^+$ populations clearly generate tumors in immunocompromised mice more efficiently than $CD133^-$ populations (33). Although CD133 is mainly expressed on the surface of proliferating cells, it has been detected on the surface of differentiated epithelial cells in a variety of tissues (http://www.proteinatlas.org/). It appears that CD133 protein expression does not change upon differentiation; however, tertiary conformational changes in differentiated colon cancer cells block the binding of AC133 antibody, suggesting that the expression of the AC133 epitope is restricted to undifferentiated stem cells (33, 38). Therefore, targeting of $CD133^+$ cells with AC133-deriviatives in human body seems to have the risk of potential side effects. However, minimal side effects are observed, suggesting that CD133 expression may be quite low in normal stem cells and plasticity of human HSCs may select a normal stem cells with a $CD133^-$ phenotype during the targeted therapies (17).

CD90 (Thy-1) is expressed on bone marrow (BM)-derived MSCs (39) and undifferentiated hESCs, whereas it is rarely expressed in normal tissue cells (40). Since CD90-positive cells from hepatocellular carcinoma cell lines have been shown to generate tumor nodules in immunodeficient mice, CD90 is also considered a marker for brain and insulinoma CSCs (41-43). EpCAM (epithelial cell adhesion molecule, CD326) is a

transmembrane glycoprotein mediating Ca^{2+}-independent homotypic cell-cell adhesion in epithelial cells. Although EpCAM (CD326) is expressed on some normal epithelial tissues and cells, it has been used as an undifferentiated hESC marker (44). EpCAM has also been found in human colon carcinoma and most adenocarcinomas, and it is involved in tumor metastases, malignant effusions, and CSCs (45). EpCAM-positive hepatocellular carcinoma and pancreatic carcinoma cells have been also suggested to function as TICs with stem/progenitor cell features (7, 46). Cripto-1 (Teratocarcinoma-derived growth factor 1) is a typical example of a common gene shared by embryonic cells and cancer cells, contributing to early embryogenesis and cancer progression. Cripto-1 has been found to be associated with the undifferentiated status of hESCs but is hardly detected in normal human cells (47). Cripto-1 also performs important roles in the formation and progression of several types of human tumors, stimulating cell proliferation, migration, epithelial-mesenchymal transition (EMT), and tumor angiogenesis. Cripto-1 expression is increased several-fold in human colon, gastric, pancreatic, lung, and breast carcinomas and enriched in a subpopulation of cancer cells with stem-like characteristics, indicating that Cripto-1 is a CSC marker (48).

PODXL-1 (Podocalyxin-like protein 1) is rarely expressed in normal tissue cells (http://www.proteinatlas.org), but highly expressed on the surface of undifferentiated hESCs (49). PODXL-1 is also expressed in hematopoietic precursor cells and leukemia (50). PODXL-1 and BMI-1 are ubiquitously expressed in small cell lung carcinoma (SCLC) due to aberrant epigenetic changes, supporting the role of PODXL-1 as a potential CSC surface marker in SCLC (51). ABCG2 (ABCP/MXR/BCRP) plays a role as a multidrug transporter in cancer drug resistance phenotype. Functional ABCG2 is highly expressed in undifferentiated hESCs (52, 53), although some controversial data are also present (54). ABCG2 protein is rarely expressed in various normal tissue cells, but it is detected in the intestine, seminal vesicle, and endothelial cells (http://www.proteinatlas.org). Side population in human lung cancer cell lines and tumors displays elevated expression of ABCG2 and is enriched with stem-like cancer cells (55). CD24 is a heavily and variably glycosylated 35-60 kDa glycosyl phosphatidylinositol (GPI)-linked sialoprotein that is rarely expressed in normal tissues except B cell precursors, neutrophils, neuronal cells, and certain epithelial cells (56). Although CD24 is expressed in human neuronal lineages, CD24 is also highly expressed in

undifferentiated hESCs (36). CD24 is detected in a wide variety of cancers and proposed as a marker for CSCs (4, 20, 57). The combination of CD24 and CD44 has been used to identify breast CSCs, because $CD44^{+}/CD24^{low}$ cells exclusively retain tumorigenic activity and display stem cell-like properties (4). CD49f (integrin α6) is highly expressed in hESCs and significantly decreased upon embryoid body formation (58). CD49f is weakly expressed in normal tissues, except in the rectum and urinary bladder (http://www.proteinatlas.org). Knockdown of CD49f in hESCs downregulates PI3K/AKT signaling and upregulates the level of p53, inducing differentiation into three germ layers (58). $CD49f^{+}$ cells are suggested as a specific HSC surface marker because they are highly efficient in generating long-term multilineage grafts (59). Targeting integrin α6 in glioblastoma stem cells (GSCs) inhibits self-renewal, proliferation, and tumor formation capacity, providing evidence that GSCs express high levels of integrin α6, which can serve not only as an enrichment marker but also as a promising anti-glioblastoma therapy (60). Notch 2 plays important roles in various developmental processes via binding with their ligand, such as Jagged (61). Notch 2 is expressed on undifferentiated hESCs and upregulated during neural differentiation of hESCs (62). It is rarely expressed in normal tissues, except in subsets of cells in the large intestine and potential endocrine cells (http://www.proteinatlas.org). Notch family is important in maintaining human NSCs via control of proliferation (63). Notch 2 is used as a CSC marker in pancreas and lung (61). CD146 is one of the most well-known surface markers for human MSCs and is also intermediately expressed on hESCs (36). Recent studies have shown that CD146 is a novel marker for highly tumorigenic cells and a potential therapeutic target in malignant rhabdoid tumor and primary sarcoma (64, 65).

CD10, CD117 and CD26 are drug target molecules approved by Federal Food and Drug Administration (FDA). CD10 (membrane metallo-endopeptidase) is a zinc-dependent metalloprotease that cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones, including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin (66). Hematopoietic progenitors expressing CD10 are considered "common lymphoid progenitors", which means they can differentiate into T, B, or natural killer cells (66). CD10 is intermediately expressed in undifferentiated hESCs and downregulated during neural differentiation of hESCs (36). CD10 is detected in human BM-

and placenta-derived MSCs (67). CD10 is rarely detected in normal tissue cells but it shows positivity in luminal membrane in the small intestine, kidney, epididymis and prostate. It is also expressed in hepatocytes (http://www.proteinatlas.org). Recent studies have shown that CD10 is a novel marker of therapeutic resistance and CSCs in head and neck squamous cell (HNSCC) and breast carcinomas (68, 69). CD117 (c-Kit) is a receptor for stem cell factor. CD117 expression is very low in normal tissue cells (http://www.proteinatlas.org), and subpopulations of hESCs (approximately 24%) are CD117-positive (36, 70). CD117 is involved in signal transduction of survival and self-renewal in various cells (71). Human epithelial ovarian cancer $CD44^{+}CD117^{+}$ cells possess the properties of CSCs that exhibit increased chemoresistance (72). CD26 (Dipeptidyl peptidase-4, DPP4) is a surface serine DPP4 expressed on different cell types, cleaves the amino-terminal dipeptide from some chemokines, including stromal-derived factor-1 (SDF-1/CXCL12), which plays important roles in HSC homing, engraftment, and mobilization. CD26 is expressed in hPSCs and HSCs (73). CD26 is rarely expressed in various normal tissue cells but it is highly expressed in kidney, small intestine, and male and female tissue cells (http://www.proteinatlas.org). Studies have shown that CD26 is a CSC marker for leukemic stem cells and colorectal CSCs (74, 75).

CSC surface markers expressed on adult stem cells, but rarely expressed on normal tissue cells

CSC surface markers that are expressed on adult stem cells, but rarely expressed on normal human cells, are summarized in Table 2. CXCR4 (CXC chemokine receptor) was originally discovered as the coreceptor for human immunodeficiency virus. CXCR4 is a potential cell surface marker for early embryonic NSCs and highly upregulated during the differentiation of hESCs to NSCs in vitro (76, 77). Extensive immunostaining of CXCR4 expression in normal human tissues is not available, but RNA expression analysis shows that CXCR4 expression is rarely expressed in many normal tissue cells, except in lymphatic organs including BM (http://www.proteinatlas.org). CXCR4 maintains a stem cell population in tamoxifen-resistant breast cancer cells and has the critical role in the metastasis of breast cancer (78, 79). CD34 was first detected on the cell surface of hematopoietic progenitor cells (80). CD34 is rarely

expressed in normal tissue, except in hematopoietic progenitor/stem cells (81). The first evidence of CSC came from studies on human AML, in which leukemic stem cells were identified as $CD34^{+}CD38^{-}$ cell subpopulation (3). CD34 is also used for isolation of TICs of squamous cell carcinomas (82).

CD271 (low-affinity nerve growth factor receptor) is rarely expressed in normal tissues, except in neural crest; it is specifically expressed in MSCs (83). CD271 has been suggested as CSC surface marker in melanoma (13). However, it is not clear whether CD271 alone is sufficient to isolate melanoma CSCs, because some melanomas metastasize in NOD/SCID $IL2R\gamma^{null}$ mice, irrespective of whether they arise from $CD271^{-}$ or $CD271^{+}$ populations (84). CD13 (alanine aminopeptidase) may regulate angiogenic signal which is related to cell morphogenesis (85). CD13 is rarely expressed in normal tissues, but highly detected in the renal tubules, intestine, exocrine pancreas, prostate, liver and gall bladder (http://www.proteinatlas.org). CD13 is a marker for MSCs isolated from various tissues (86). CD13 is suggested as a putative marker for liver CSCs (87). CD56 (Neural cell adhesion molecule) is a homophilic binding glycoprotein expressed on the surface of neurons, glia, skeletal muscle, and natural killer (NK) cells. CD56 is rarely expressed in normal tissue cells, except in the central and peripheral nerves (88). CD56 is a marker for MSCs and small-cell lung CSCs (89). CD105 (endoglin) is a member of the transforming growth factor β (TGF) receptor family that binds TGF-β1 and -β3 on human endothelial cells (90). CD105 has been known as a cell surface marker for MSCs (91). Tumoral CD105 has been described as a new CSC marker of renal cell carcinomas (92). LGR5 (Leucine-rich repeat-containing G-protein coupled receptor 5) is a member of G protein-coupled receptor which is not expressed on hESCs (93). Since LGR5 is discovered as an adult stem cell marker in small intestine (94), LGR5 is considered as a biomarker of adult stem cells in multiple epithelia (95). LGR5 is rarely expressed in various normal tissue cells, although it is detected in the brain, gastrointestinal and female tissues (http://www.proteinatlas.org). LGR5 is a CSC marker in mouse intestinal cancers (96). LGR5 has been also suggested as a CSC maker for human colon and colorectal cancers (97, 98).

CD114 (colony stimulating factor 3 receptor) is a cytokine receptor and plays an important role in granulopoiesis during the inflammatory process. It is present on precursor

cells in the BM, and initiates cell proliferation and differentiation into mature granulocytes and macrophages in response to stimulation by G-CSF (99). CD114 is rarely expressed in normal tissue cells, except in the brain, placenta, heart muscle, testis and skin (http://www.proteinatlas.org). CD114 has been identified as a potential marker for CSCs in neural crest-derived tumors (100, 101). CD54 (Intercellular adhesion molecule 1) is related to cell-cell interaction (102). CD54 is not expressed in hESCs but weakly expressed in MSCs (103). CD54 is rarely expressed in many normal tissue cells, but highly detected in the lung, kidney and lymphoid organs (http://www.proteinatlas.org). CD54 is also used for isolation of gastric cancer stem cells (104). CXCR1 (Chemokine receptor 1) and CXCR2 (Chemokine receptor 2) are integral membrane proteins, which specifically bind and respond to cytokines of the CXC chemokine family. These receptors bind to IL8 with high affinity, and transduces signaling through a G-protein activated second messenger system (105). CXCR1 only shows moderate membranous positivity in a subset of cells in blood vessels (http://www.proteinatlas.org). CXCR1 and CXCR2 are not only expressed on the surface of MSCs (106), but also expressed on breast and pancreas CSCs (107, 108). TIM-3 (T-cell immunoglobulin domain and mucin domain-3) is an activation-induced inhibitory molecule involved in immune tolerance. TIM-3 is only expressed in a subset of lymphoid cells in normal tissues (http://www.proteinatlas.org). TIM-3 is not expressed on the surface of normal HSCs but highly expressed on leukemic stem cells in most type of AML (109). CD55 (decay-accelerating factor) is not detected in normal tissues, except in the ovary, lung, placenta, adrenal gland and salivary gland (http://www.proteinatlas.org). CD55 may be a novel surface marker for breast CSCs because a small population of cells with strong CD55 expression is sufficient to predict poor prognosis in breast cancer patients (110). DLL4 (delta-like ligand 4) is an important component of the Notch pathway and contributes to stem cell self-renewal and vascular development. Notch signaling in stem cells and progenitors is activated by DLL1 and DLL4 ligands and is required for maintenance of intestinal progenitor and stem cells (111). Inhibiting human DLL4 in the tumors, either alone or in combination with the chemotherapeutic agent irinotecan, reduces CSC frequency because DLL4 blockade inhibits TIC frequency (112, 113).

CD20 and CD96 are expressed in B and T cell lineage cells, respectively, rather than stem

cells. The function of CD20 is not clear during B-cell development (114). CD20 is not expressed in normal tissues except in the lymphoid organs and skin (http://www.proteinatlas.org). Even though CD20 expression is not distinguished between normal B-lymphocytes and malignant melanoma, CD20 is used as a marker for melanoma (115). Melanomas contain a $CD20^+$ subpopulation of melanoma cells that contributes to melanoma heterogeneity and tumorigenesis (116). CD96 functions as a T cell-specific receptor (117). CD96 is a transmembrane glycoprotein on human and mouse T and NK cells (118). CD96 is not expressed by a majority of cells in normal HSCs, but it is frequently expressed on leukemic stem cells (119, 120).

CSC surface markers expressed on both stem cells and normal tissue cells

CSC surface markers that are expressed on both hESCs and normal tissue cells are summarized in Table 3. CD29 (Integrin β1) is a cell adhesion molecule that mediates interactions between adhesion molecules on adjacent cells and/or the extracellular matrix (121). CD29 is highly expressed in both hESCs and MSCs while it also ubiquitously expressed in various normal tissues (http://www.proteinatlas.org) (36, 122). CD29 has been suggested to be a cell surface marker for breast CSCs because $CD29^+CD49f^+$ cell population displays CSC activity in allograft-nude mice (123). CD9 (MRP-1) is a tetraspan family glycoprotein which has been shown to modulate cellular adhesion, migration, and proliferation (124). CD9 has been used as a cell surface marker of undifferentiated hESCs (70) and adipose-derived MSCs (125). CD9 protein expression is detected in a majority of normal tissues, but its expression is negative or weak in the gall bladder, liver, and lymphoid tissues (http://www.proteinatlas.org). CD9 is a useful positive-selection marker for identification of CSCs in human B-acute lymphoblastic leukemia cells (B-ALL), and links to several signaling pathways and epigenetic modification for regulating the CSC properties of B-ALL (126). CD166 (activated leukocyte cell adhesion molecule) is a type I membrane glycoprotein which is a member of the immunoglobulin superfamily. Its expression is detected in many epithelial cells (http://www.proteinatlas.org). CD166 is weakly expressed in undifferentiated hESCs (36) and it is a marker for multipotential human adipose-derived stromal stem cells and intestinal stem cells (127, 128). CD166 is also a marker of colorectal CSCs (129). CD166 has been

identified as an "inert" CSC surface marker for non-small cell lung cancer (NSCLC), but a controversial study is also present (9, 130).

CSC surface markers that are expressed on both adult stem cells and normal tissues are also summarized in Table 3. CD44, a hyaluronic acid receptor, is one of the most frequently studied markers in various cancer cells. CD44 is a multi-structural and multi-functional cell surface molecule, whose role is primarily governed by various post-translational modifications (131). CD44 standard (CD44s) is an 85-90-kDa transmembrane glycoprotein, containing 10 standard exons, whereas tissue-specific splice variants (CD44v1-10) contain the standard set and combinations of the 10 variable exons. The CD44 family has many isoforms that are expressed by alternative splicing of the pre-mRNA (131). Its function is implicated in cell adhesion and migration, but the prominent role of CD44 is to bind to hyaluronic acid in the extracellular matrices. CD44 has been detected in human HSCs (132), MSCs (91), and adipose-derived stem cells (133). CD44 has been also used extensively in combination or with other putative markers to isolate CSCs from various solid tumors (131, 134). CD44s is ubiquitously expressed in many normal cell types; however, its significance as a CSC marker may be limited (135). Recent studies suggest that conflicting results may be attributed to the expression of alternatively spliced variants. In this regard, CD44 variant 9 (CD44v9) has emerged as a novel marker of cancer stemness in a variety of solid tumors (136-139). Another variant CD44v8-10 whose expression is low in normal tissues also appears to be a cancer-specific marker for gastric CSCs (140). The other variants of CD44 have been also suggested for CSC markers in various cancers (131).

ABCB5 is an ATP-binding cassette transporter and a P-glycoprotein family member, principally expressed in physiological skins and human malignant melanomas. It is expressed on normal liver and limbal stem cells (141). ABCB5 shows weak and moderate cytoplasmic staining in a majority of normal tissues (http://www.proteinatlas.org). Because $ABCB5^{+}$ subpopulations show self-renewal and differentiation capacity, $ABCB5^{+}$ tumor cells have been suggested as melanoma-initiating cells (142). Notch 3 is important for maintaining human NSCs via control of cell proliferation (63). Notch 3 protein is ubiquitously expressed in many normal tissue cells including appendix, gallbladder and urinary bladder (http://www.proteinatlas.org). However, Notch 3 is suggested for a CSC marker in pancreas

and lung cancers (61). CD123 is an interleukin 3 specific subunit of a heterodimeric cytokine receptor, which is highly expressed in AML. IL-3 treatment increases the proliferation of AML (143). CD123 is ubiquitously expressed in normal human tissues (http://www.proteinatlas.org). CD123 is a well-known target for the therapy of leukemia, because it is not expressed on normal HSCs but highly expressed on leukemic stem cells (144).

Similarities between CSC surface markers and stem cell surface markers

Among 40 CSC surface markers described above, most of them are expressed on both CSCs and normal stem cells, suggesting that there is a high level of similarity between CSC surface markers and stem cell surface markers. The idea that cancers arise from residual embryonic tissues appeared in the early 19th century and was formally published by Durante and Conheim as the "embryonic rest hypothesis of cancer development" (145, 146). This hypothesis states that remnants of embryonic tissue remain in adult organism, and cancers arise from the remaining embryonic cells (145, 146). Based on the hypothesis, adult stem cells would be leftover ESCs in adult tissues after birth. Interestingly, cancer and embryonic cells show very similar histological morphologies and have many common features, such as reduced contact inhibition, high proliferation rate, tissue invasion ability, anaerobic metabolism, dedifferentiation status, evasion of immune destruction, secretion of angiogenic factors, and expression of embryonic genes. In the 1970s, researchers found that rabbits immunized with mouse embryos create antibodies that cross-reacted with 72 different mouse tumors (147). Antibodies produced against human embryos also recognize a variety of human tumors, including lung, skin, bronchial, renal, colonic, hepatic and breast (148). Immunization with embryonic cells has similar results; immunized mice make antibodies that recognize both tumors and embryos (149, 150). These findings led to the idea that animals or humans vaccinated with embryonic tissues might trigger an immune response against cancer and prevent cancer progression. Interestingly, vaccination with embryonic cells does not show cross-reactivity with various adult tissues, except skin (145). These and following studies provide the concept about "oncofetal antigens" that are typically present during embryonic and fetal development, but are found only in cancerous tissues in adults (150).

The relationship between cancer and embryonic tissues/cells has attracted a lot of attention again after the development of hESCs and CSCs. Li *et al.,* 2009 reported that vaccination of mice with hESCs results in strong immune responses against colon carcinoma cells without autoimmune responses (151). Mice vaccinated with mouse ESCs induce obvious anti-tumor immunity, which protects them from the formation and development of lung cancer (152). Mice vaccinated with mouse ESC along with a source of granulocyte macrophage-colony stimulating factor also suppress the development of lung cancer induced by the combination of carcinogen administration and chronic pulmonary inflammation (153). These findings suggest the concept that ESCs have oncofetal antigens, which are also present on cancer cells. The concept about oncofetal antigens would be expended to adult stem cells because adult stem cells are considered as leftover ESCs in adult tissues. Global analysis of gene expression networks further suggest that core pluripotency genes, such as MYC, NANOG, OCT4, and SOX2 are fundamental gene circuits shared by both ESCs and cancers (154, 155). Almost half of the genes that are transcriptionally upregulated, as a result of genomic aberrations present in hESCs, are also tightly linked to the expression of cancer genes (156, 157).

The basic similarities between hESCs and CSCs are that both have pluripotency or multipotency and express the same oncofetal antigens, such as SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, EpCAM, and Cripto. When injected into immunodeficient mice, both can also create teratoma tumors. The other common characteristics between ESCs and CSCs are indefinite self-renewal, high proliferation potential, high nuclear to cytoplasmic ratio, and increased expression of anti-apoptotic genes (158). The activation of ESC-like gene expression in adult cells is considered to endow self-renewal to CSCs (159). CSCs also share common signaling pathways, such as Notch, Sonic hedgehog, Wnt, and Fibroblast growth factor-2 that also regulate hESCs (158, 159). Thus, hESCs and CSC have high potential to have the same cell surface markers (Fig.1). Until now, approximately 40 CSC surface markers have been identified (Table 1-3). Among the 40 CSC markers, 35 markers (approximately 88%) are also expressed on normal embryonic or adult stem cells, which demonstrate the basic similarities between CSCs and normal stem cells.

Conclusion and future perspectives

We summarize 40 CSC surface markers in this review, although some known surface markers are not accurate and need further studies. To better isolate specific CSCs from various heterogeneous tumors, more functional markers should be developed. To isolate functional CSCs, there is a need to search for more specific surface markers or to use many surface markers in combination. We classify currently known 40 CSC surface markers into 3 different categories, depending on their expression on hESCs, adult stem cells, and normal tissue cells. Among the 40 CSC markers, approximately 83% (33 out of 40 CSC markers) are rarely expressed on normal tissue cells (Table 1-3). We believe that the CSC surface markers will have potential usefulness as therapeutic targets against CSCs due to low cross reactivity to normal tissue cells. As expected, 9 of them were already approved as drug target molecules by FDA. Seven CSC surface markers are ubiquitously expressed on normal tissue cells (Table 3), which may lead to side effects when they are targeted for elimination. For example, CD44s is ubiquitously expressed in many normal cell types, which may cause side effects in CD44s-targeted therapies. According to recent studies, however, variant CD44v8-10 is a bona fide CSC-specific marker (136-140). Interestingly, the variant CD44v8-10 is weakly expressed in normal tissues, suggesting that the ambiguity regarding functional aspects of CD44 in CSC identity largely attributes to the expression of alternatively spliced variants. In this regard, functional epitopes on some CSC surface markers should be extensively defined for specific detection of CSCs in future studies. Most of CSCs were isolated by using monoclonal or polyclonal antibodies. Recent studies have shown that general protein expression is not sufficient to isolate specific CSCs from heterogeneous populations (38, 136-140). In the case of CD133, the expression of AC133 epitope on CD133 protein is only restricted to undifferentiated stem cells (38), suggesting that CSC-specific epitopes are necessary to analyze functional CSC activity. CSC-specific epitopes may be also present or absent depending on the CD44 splice variant, which may generate some conflicting data in CD44-expressed cancers. Many commercially available antibodies are generated against synthetic peptides from target proteins, instead of real tertiary and native forms of target proteins, and the use of the antibodies may lead to misinterpretation about the functional CSCs. Therefore, the development of many antibodies recognizing CSC-specific functional

epitopes is necessary to overcome the current ambiguity of some CSC surface markers.

Identification of a novel CSC marker is very difficult because CSCs are generally rare in tumor tissues (1). Therefore, identifying novel surface markers on normal stem cells will be an alternative approach to find novel surface markers on CSCs. However, adult stem cells are very rare in mature tissues, so isolating these cells from an adult tissue is challenging, and culture methods to expand their numbers up to the numbers required is another challenge. When surface markers on adult stem cells are utilized as therapeutic targets against CSCs, furthermore, there may be the possibility to eliminate normal adult stem cells and impair the normal process of tissue regeneration. Contrary to adult stem cells, hESCs can be grown relatively easily in culture. Among 40 CSC markers, 21 CSC surface markers (approximately 53%) are expressed on hESCs as well. Many of these surface markers originate from surface markers on undifferentiated hESCs. These surface markers may be candidates as CSC markers because surface markers of undifferentiated hESCs have oncofetal characteristics and are rarely expressed on normal tissue cells (Table 1). By using a modified decoy immunization strategy, we generated 37 MAbs which bind to undifferentiated hESCs but weakly or not at all to differentiated hESCs or differentiated primary cells (160). By using the MAbs, we found that cell surface-expressed E1B-AP5 and BAP31 are novel surface markers on undifferentiated hESCs (161, 162). Interestingly, cell surface E1B-AP5 and BAP31 are also expressed on some cancer cell lines, while they are not expressed on normal differentiated cells (161, 162), suggesting that these kinds of hESC surface marker deserve to be studied as potential CSC surface markers. Thus, finding novel surface markers on undifferentiated hESCs will be an attractive alternative to screen novel CSC surface markers. A proposed strategy for the identification of novel CSC surface markers by using hESC/hPSC-specific MAbs is presented in Fig.1.

ACKNOWLEDGEMENTS

We thank Profs Yonghyun Kim and Hee Chul Lee for their comments and careful proofreading. This study was supported by the National Research Foundation of Korea (2016-903249 and 2016-008610).

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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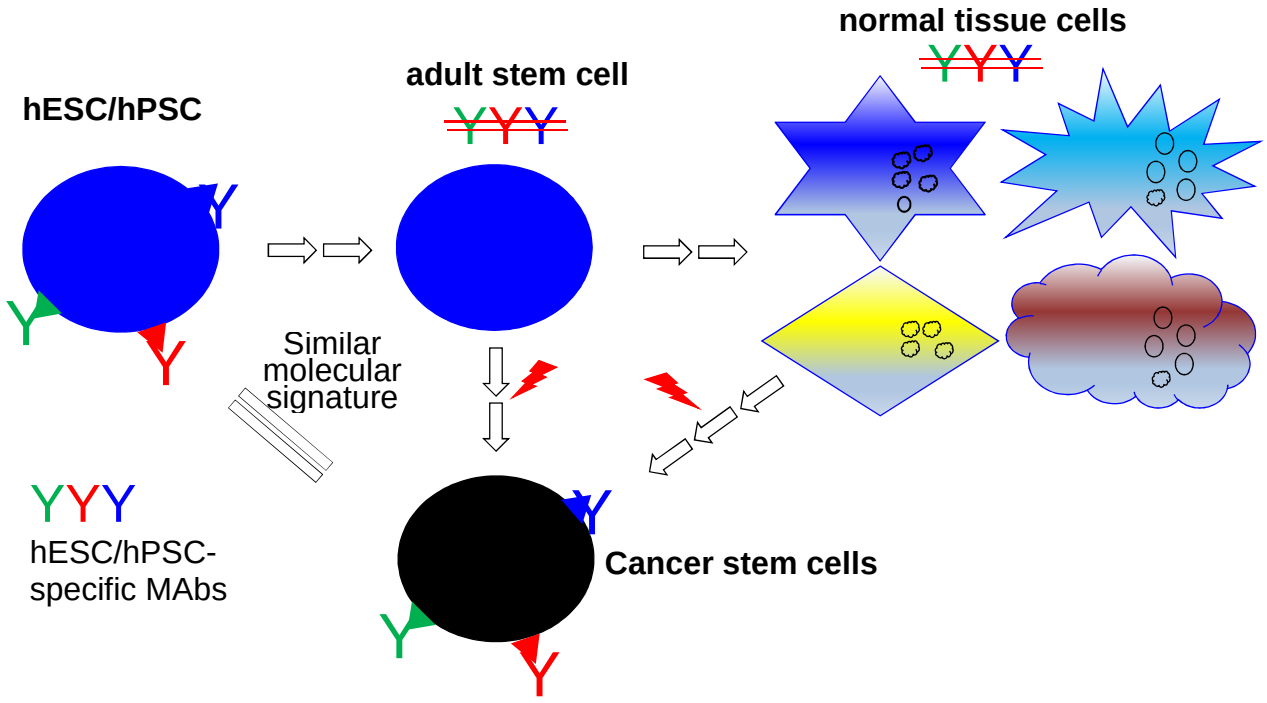

Fig. 1. Proposed strategy for the identification of novel CSC surface markers by using hESC/hPSC-specific MAbs. An overall scheme showing a normal cellular hierarchy of embryonic stem cells (hESC/hPSC), adult stem cell and differentaited normal tissue cells is shown. Cancer stem cells can be derived from hESC/hPSC, adult stem cell and normal tissues cells. MAbs specific to undifferentiated hESCs/hPSCs, but not to adult stem cells and normal tissue cells, will be attractive tools to find novel CSC surface markers because the antigens recognized by the MAbs is highly likely to be present on CSCs, but not on normal tissue cells, in adults.

Table 1. CSC surface markers that are expressed on hESCs but rarely expressed in normal tissue cells

CSC surface marker	Origin and function	Expression in hESC/hPSC	Expression in adult stem cell	Expression in normal tissues/cells	Expression in CSCs	Ref.
SSEA3	hESC marker	yes	mesenchymal	rare	teratocarcinoma, breast	(22, 23, 26)
SSEA4	hESC marker	yes	mesenchymal, cardiac	rare	teratocarcinoma, breast	(24-26)
TRA-1-60	hESC marker	yes	NA*	rare	teratocarcinoma, breast, prostate	(28, 29)
TRA-1-81	hESC marker	yes	NA	rare	teratocarcinoma, breast	(28)
SSEA1	mouse ESC marker	yes (mouse)	cardiac	rare	teratocarcinoma, renal, lung	(30-32)
CD133 (AC133)	marker for hematopoietic stem cells.	yes	hematopoietic neural prostate	rare (proliferative cell)	breast, prostate, colon, glioma, liver, lung, ovary,	(33-38)
CD90 (Thy-1)	signal transduction / cell adhesion	yes	mesenchymal, cardiac	rare (T-cell, neuron)	brain, liver	(39-43)
CD326 (EpCAM)	cell adhesion, signal transduction	yes	no	rare (epithelial cell)	colon, pancreas, liver	(7, 44-46)
Cripto-1 (TDGF1)	self-renewal /survival in esc	yes	NA	rare (pancreas, hippocampus)	breast, colon, lung	(47, 48)
PODXL-1 (Podocalyxin-like protein 1)	ligand for L-selectin	yes	mesenchymal hematopoietic	rare (podocyte)	leukemia, breast, pancreas, lung	(49-51)
ABCG2	ATP-binding cassette transporter	yes	hematopoietic muscle neural	rare (myogenic)	lung, breast, brain	(53-55)
CD24	B cell proliferation	yes	intestinal	rare (B lymphoid, neural)	breast, gastric, pancreas	(4, 36, 57)
CD49f (Integrin α6)	cell adhesion	yes	hematopoietic	rare (rectum, urinary bladder)	glioma	(58-60)
Notch2	signal transduction	yes	neural	rare (subset in large intestine)	pancreas, lung	(61-63)
CD146 (MCAM)	melanoma cell adhesion molecule	yes	mesenchymal	rare (endothelial, ganglion cell)	rhabdoid tumor, sarcoma	(36, 64, 65)
CD10 (Neprilysin)	metallo-endopeptidase, FDA-approved target	yes	mesenchymal	rare (glandular cells in some tissues)	breast, head and neck	(36, 66-69)
CD117 (c-KIT)	receptor for stem cell factor, FDA-approved target	yes	mesenchymal cardiac	rare (myeloid)	ovary	(36, 70-72)
CD26 (DPP-4)	dipeptidyl peptidase iv, FDA-approved target	yes	hematopoietic	rare (intestine, kidney, male, female tissues, activated T, B, NK cells)	colorectal, leukemia	(73-75)

*not available

Table 2. CSC surface markers that are expressed adult stem cells but rarely expressed on normal tissue cells

CSC surface marker	Origin and function	Expression in hESC/hPSC	Expression in adult stem cell	Expression in normal tissue/cells	Expression in CSCs	Ref.
CXCR4	receptor for chemokine, FDA-approved target	no	neural	rare (lymphoid)	breast, brain, pancreas	(76-79)
CD34	cell adhesion	no	hematopoietic	rare (lymphoid)	leukemia, squamous cell carcinoma	(3, 80-82)
CD271	nerve growth factor receptor	no	mesenchymal	rare (neural crest)	melanoma, head and neck	(13, 83, 84)
CD13 (Alanine aminopeptidase)	marker for kidney disease	no	mesenchymal	rare (myeloid)	liver	(85-87)
CD56 (NCAM)	cell adhesion	no	mesenchymal	rare (lymphoid)	lung	(88, 89)
CD105 (Endoglin)	coreceptor for TGF-β	no	mesenchymal	rare (endothelial)	renal	(90-92)
LGR5	cell adhesion	no	intestinal, kidney, stomach, hair follicle	rare (brain, intestine, female tissues)	intestinal, colorectal	(93-98)
CD114 (CSF3R)	colony stimulating factor 3 receptor, FDA-approved target	no	neural crest, BM-derived precursors	rare (placenta, BM, brain, heart muscle, skin)	neuroblastoma	(99-101)
CD54 (ICAM-1)	cell adhesion, FDA-approved target	no	mesenchymal	rare (endothelial cell)	gastric	(102-104)
CXCR1, 2	receptor for chemokine	NA*	mesenchymal	rare (spleen, leucocyte subset)	breast, pancreas	(105-108)
TIM-3 (HAVCR2)	immune checkpoint receptor	NA	NA	rare (lymphoid)	leukemia	(109)
CD55 (DAF)	inhibitor of complement	NA	NA	rare (lymphoid)	breast	(110, 163)
DLL4 (Delta-like ligand 4)	Notch ligand	NA	intestinal	rare (intestine, liver, gall bladder and renal tubuli, Purkinje and glandular cells)	colorectal, ovarian	(111-113)
CD20 (MS4A1)	B cell lineage, FDA-approved target	no	no	rare (lymphoid)	melanoma	(114-116, 164, 165)
CD96	T cell-specific receptor	NA	no	rare (weak in lymphoid)	leukemia	(117-120, 166)

*not available

Table 3. CSC surface markers that are expressed on both stem cells and normal tissue cells

CSC surface marker	Origin and function	Expression in hESC/hPSC	Expression in adult stem cell	Expression in normal tissue cells	Expression in CSCs	Ref.
CD29 (Integrin β1)	Cell adhesion, FDA-approved target	yes	mesenchymal	ubiquitously	breast, colon	(36, 121-123)
CD9	Cell adhesion	yes	adipose-derived mesenchymal	many tissues (except gall bladder, liver, lymphoid tissues)	leukemia	(70, 124-126)
CD166 (ALCAM)	Cell-cell/cell-matrix interaction	yes (weak)	adipose, intestine	many epithelial cells	colorectal, lung	(9, 36, 127-130)
CD44 variants	Hyaluronic acid receptor, FDA-approved target	no	hematopoietic Adipose mesenchymal	most epithelial and lymphatic tissues	HNSCC, breast, colon, liver, ovarian, pancreas, gastric	(91, 131-140)
ABCB5	ABC transporter	NA*	limbal	majority of normal tissues (weak, moderate)	melanoma	(141, 142)
Notch3	Signal transduction	NA	neural	many tissues	pancreas, lung	(61, 63)
CD123 (IL-3R)	Receptor for IL-3	NA	no	majority of normal tissues	leukemia	(143, 144)

*not available