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Invited Mini Review

Cancer stem cell surface markers on normal stem cells

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The cancer stem cell (CSC) hypothesis has captured the attention of many scientists. It is believed that elimination of CSCs could possibly eradicate the whole cancer. CSC surface markers provide molecular targeted therapies for various cancers, using therapeutic antibodies specific for the CSC surface markers. Various CSC surface markers have been identified and published. Interestingly, most of the markers used to identify CSCs are derived from surface markers present on human embryonic stem cells (hESCs) or adult stem cells. In this review, we classify the 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 remaining CSC surface markers are considerably expressed even in normal tissue cells, and some of them have been extensively validated as CSC surface markers by various research groups. We discuss the significance of the categorized CSC surface markers, and provide insight into why surface markers on hESCs are an attractive source to find novel surface markers on CSCs. [BMB Reports 2017; 50(6): 285-298]

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 having a self-renewal capacity and potential to differentiate into multiple cell lineages (1). Rare subsets of cancer cells with stem-like properties, referred to as cancer stem cells (CSCs) or tumor initiating cells (TICs), are 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, whereas the major abundant subset of $CD34^+CD38^+$cells was ineffective (3). The results demonstrates for the first time the existence of CSCs in liquid tumors, and encouraged 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, including 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 these cells will lead to the disappearance of entire tumors, based on the concept that the sole source of tumor self-renewal is the CSC. Since CSCs were identified on the basis of their cell surface molecules, specific antibodies/immunotoxins against the surface molecules have also been successfully developed to selectively eradicate CSCs (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 the 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 known normal embryonic or adult stem cell surface markers (1, 2, 19-21). The similarity of cell surface markers suggests that CSCs predominantly originate from normal stem cells via the accumulation of epigenetic and genetic alterations (20). In this review, the currently published 40 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 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 are weakly or rarely expressed on normal tissue cells (Table 2). The third group of

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Table 1. CSC surface markers 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.

CSC surface markers are expressed on hESCs and/or adult stem cells, and are also considerably expressed on various normal tissue cells (Table 3). In the tables, the histological data of some CSC surface markers not been published before, originates from the human protein atlas (http://www.proteinatlas.org/). CD133 is the most frequently studied CSC surface marker in various cancers, and specific antibodies/immunotoxins against CD133 have been successfully developed for their selective eradication (14, 17). CD133 expression is detected in 22 of 82 cell types from 44 normal human tissues

Table 2. CSC surface markers expressed on 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.

(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 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, and 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 are expressed on breast cancer cells and breast CSCs (26). TRA-1-60 and TRA-1-81 antigens, expressed on podocalyxin in human pluripotent stem cells (hPSCs) (27), are associated 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 surface marker for neural stem cells (NSCs), and SSEA-1^+ cells from brain tumors show properties of brain tumor stem cells (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

Table 3. CSC surface markers 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.

with five transmembrane domains and two large extracellular loops (33). The exact function of CD133 still remains unknown, but it seems to organize 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 is downregulated upon the differentiation of hESCs, suggesting that CD133 expression is restricted to undifferentiated hESCs (36). CD133 is one of the most frequently studied surface markers in solid cancers (33). The CD133 marker has identified 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 also 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). Targeting $CD133^+$ cells with AC133-derivatives in the human body also shows minimal side effects, suggesting that CD133 expression may be quite low in normal stem cells, and the 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 $CD90^+$ cells from hepatocellular carcinoma cell lines are capable of generating 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 is expressed on some normal epithelial tissues and cells, it has been used as an undifferentiated hESC marker (44). EpCAM is also found in most adenocarcinomas, and is involved in tumor metastases and CSCs (45). $EpCAM^+$ hepatocellular carcinoma and pancreatic carcinoma cells have been suggested to function as TICs with stem/progenitor cell features (7, 46). Cripto-1 (Teratocarcinoma-derived growth factor 1) is one of many common genes shared by both embryonic cells and cancer cells, and it contributes to early embryogenesis and cancer progression. Cripto-1 is associated with undifferentiated hESCs, but is hardly detected in normal human cells (47). Cripto-1 also has important functions in many human tumors, promoting cancer cell migration, proliferation, epithelial-mesenchymal transition (EMT), and angiogenesis. Cripto-1 expression is increased several-fold in human colon, gastric, pancreatic, lung, and breast carcinomas, and can be enriched from 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 is 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). The ATP-binding cassette sub-family G member 2 (ABCG2/ABCP/MXR/BCRP) functions as a multidrug transporter in cancer drug resistance phenotypes. Although functional ABCG2 is highly expressed in undifferentiated hESCs (52, 53), some controversial data are also present (54). ABCG2 protein is rarely expressed in normal tissue cells, but some amount 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, 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, it is highly expressed in undifferentiated hESCs (36). Since CD24 is detected in a wide variety of cancers, it is proposed as a marker for CSCs (4, 20, 57). The combination of CD24 and CD44 is used to identify breast CSCs, since $CD44^+/CD24^{low}$ cells exclusively retain tumorigenic activity and display stem cell-like properties (4). CD49f (integrin α6) is highly expressed in hESCs, and significantly decreases 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 HSC population because they are highly efficient in generating long-term multilineage grafts (59). Targeting CD49f in glioblastoma stem cells (GSCs) suppresses self-renewal, proliferation, and tumor formation capacity, providing evidence that GSCs express high levels of CD49f, which serve not only as an isolation marker, but also as an anti-glioblastoma target (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/). The 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 reveal that CD146 is a novel marker for highly tumorigenic cells, and is a potential therapeutic target in malignant rhabdoid tumor and primary sarcoma (64, 65).

CD10, CD117 and CD26 are drug target molecules approved by the Federal Food and Drug Administration (FDA). CD10 (membrane metallo-endopeptidase) is a membrane-bound metallopeptidase that inactivates various peptide hormones, including glucagon, substance P, oxytocin, and bradykinin (66). $CD10^+$ hematopoietic progenitors are "common lymphoid progenitors", which can differentiate into T, B, or natural killer cells (66). CD10 is intermediately expressed in undifferentiated hESCs, and is downregulated during neural differentiation of hESCs (36). CD10 is detected in human BM- and placenta-derived MSCs (67), but is rarely detected in normal tissue cells. However, it shows positivity in the luminal membrane in the small intestine, kidney, epididymis and prostate, and is also expressed in hepatocytes (http://www.proteinatlas.org/). Recent studies have shown that in head and neck squamous cell (HNSCC) and breast carcinomas, CD10 is a novel marker for therapeutic resistance and CSCs (68, 69). CD117 (c-Kit) is a receptor for stem cell factor, having very low expression in normal tissue cells (http://www.proteinatlas.org/). 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 properties of CSCs, exhibiting increased chemoresistance (72). CD26 (dipeptidyl peptidase-4, DPP4) is a surface serine DPP4 expressed on different cell types, and is involved in cleaving the amino-terminal dipeptide from some chemokines, including C-X-C motif chemokine ligand 12/stromal cell-derived factor-1 (CXCL12/SDF-1), which has important roles in HSC engraftment, mobilization and homing. CD26 is expressed in hPSCs and HSCs (73), 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 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 a coreceptor for the human immunodeficiency virus. CXCR4 is a potential cell surface marker for early embryonic NSCs, and is highly upregulated during the differentiation of hESCs to NSCs *in vitro* (76, 77). Extensive immunostaining of CXCR4 expression in normal human tissues is unavailable, but RNA expression analysis reveals 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 a critical role in the metastasis of breast cancer (78, 79). CD34, first detected on the cell surface of hematopoietic progenitor cells (80), 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 a

$CD34^+CD38^-$ cell subpopulation (3). CD34 is also required for the isolation of TICs of squamous cell carcinomas (82).

CD271 (low-affinity nerve growth factor receptor) is specifically expressed in MSCs, and is rarely expressed in normal tissues, except in neural crest (83). CD271 has been suggested as a 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 the angiogenic signal, which is related to cell morphogenesis (85). CD13 is rarely expressed in normal tissues, but highly detected in renal tubules, intestine, exocrine pancreas, prostate, liver and gall bladder (http://www.proteinatlas.org/). It is a marker for MSCs isolated from various tissues (86), and is a suggested putative marker for liver CSCs (87). CD56 (neural cell adhesion molecule) is a membrane glycoprotein expressed on the surface of neurons, skeletal muscle and natural killer (NK) cells, and is a marker for MSCs and small-cell lung CSCs (88). CD56 is rarely expressed in normal tissue cells, except in the central and peripheral nerves (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). 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, and is not expressed on hESCs (93). Discovered as an adult stem cell marker in the small intestine (94), LGR5 is considered as a biomarker of adult stem cells in multiple epithelia (95). It 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), and has also been 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); it is not expressed in hESCs, but is weakly expressed in MSCs (103). Although rarely expressed in many normal tissue cells, CD54 is highly detected in the lung, kidney and lymphoid organs (http://www.proteinatlas.org/). CD54 is also used in the isolation of gastric CSCs (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 have a high binding affinity to IL8, and transduce signaling through a G-protein activated second messenger system (105). CXCR1 shows moderate membranous positivity in a subset of cells in the blood vessels (http://www.proteinatlas.org/). CXCR1 and CXCR2 are not only expressed on the surface of MSCs (106), but are 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 is highly expressed on leukemic stem cells in most types 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, since a small population of cells with CD55 expression is correlated to poor prognosis in breast cancer patients (110). DLL4 (delta-like ligand 4) serves as a ligand for Notch signaling and promotes stem cell self-renewal and vascular development. Notch signaling is necessary for maintaining intestinal progenitor and stem cells (111). Inhibiting human DLL4 in the tumors reduces the CSC frequency because of the inhibition of TIC frequency by the DLL4 blockade (112, 113).

CD20 and CD96 are expressed in B and T lineage cells, respectively, rather than in 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); it 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 the interactions between adhesion molecules on adjacent cells and/or the extracellular matrix (121). It is highly expressed in both hESCs and MSCs, and is also ubiquitously expressed in various normal tissues (http://www.proteinatlas.org/) (36, 122). CD29 has been suggested as a cell surface marker for breast CSCs, because the $CD29^+CD49f^+$ cell population displays CSC activity in allograft-nude mice (123). CD9 (MRP-1) is a

tetraspan family glycoprotein which modulates cellular adhesion, migration, and proliferation (124). CD9 is 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/). However, it is a useful marker to identify CSCs in human B-acute lymphoblastic leukemia cells (B-ALL), and is linked to several signaling pathways involved in 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 is a marker for multipotential human adipose-derived stromal stem cells and intestinal stem cells (127, 128). Although CD166 is a marker of colorectal CSCs (129), and has also been identified as an "inert" CSC surface marker for non-small cell lung cancer (NSCLC), some controversial studies are also present (9, 130).

CSC surface markers expressed on both adult stem cells and normal tissues are 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). The CD44 family has many isoforms that are expressed by alternative splicing of the pre-mRNA (131). CD44 standard (CD44s) is an 85-90-kDa transmembrane glycoprotein with basic 10 standard exons, whereas tissue-specific splice variants (CD44v1-10) consist of the standard set and combinations of the 10 variable exons. Its function is implicated in cell adhesion and migration, but a 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), and has been extensively used 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). Other variants of CD44 have also been suggested as 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. 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 by controlling 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 as 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, since it is not expressed on normal HSCs but is highly expressed on leukemic stem cells (144).

SIMILARITIES BETWEEN CSC SURFACE MARKERS AND STEM CELL SURFACE MARKERS

Most of the 40 CSC surface markers described above 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 these 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 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 shows 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 subsequent studies provide the concept about “oncofetal antigens” that are typically present only during embryonic and fetal development, but are found in cancerous tissues in adults (150).

The relationship between cancer and embryonic tissues/cells has attracted a lot of attention 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, cocultured with STO fibroblasts expressing granulocyte macrophage-CSF, also suppress lung cancer development induced by 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 being expended to adult stem cells is 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 NANOG, OCT4, SOX2, and MYC, are primary gene sets shared by both ESCs and cancers (154, 155). Almost half of the genes that are upregulated as a result of genomic alterations in hESCs, are also closely 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 immuno-deficient mice, both are capable of generating teratoma tumors. Both ESCs and CSCs also have other common characteristics, such as high proliferation potential, indefinite self-renewal, 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). Analysis of signaling molecules in CSCs reveals that CSCs also contain common signaling, such as Wnt-, Notch-, Sonic hedgehog- and Fibroblast growth factor-2-signaling that regulate the hESCs as well (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), of which 35 markers (approximately 88%) are also expressed on normal embryonic or adult stem cells, thus demonstrating 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 are needed. To isolate functional CSCs, there is a need to search for more specific surface markers, or use multiple surface markers in combination. We classify the currently known 40 CSC surface markers into 3 different categories, depending on their expression on hESCs, adult stem cells, and normal tissue cells. Of 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 have potential usefulness as therapeutic targets against CSCs due to their low cross reactivity to normal tissue cells. As expected, 9 of these are 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, the 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

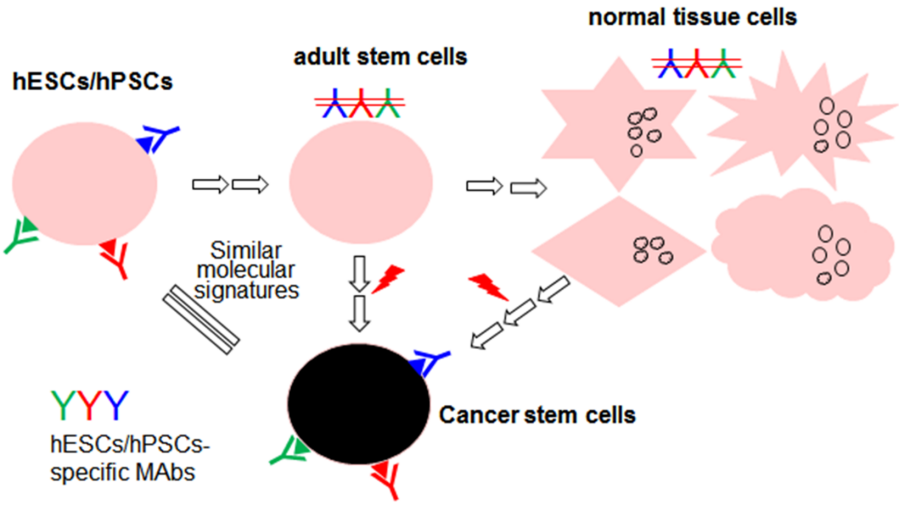

Fig. 1. Proposed strategy for the identification of novel CSC surface markers by using hESCs/hPSCs-specific MAbs. Shown is an overall scheme showing a normal cellular hierarchy of embryonic stem cells (hESCs/hPSCs), adult stem cells, and differentaited normal tissue cells. Cancer stem cells can be derived from hESCs/hPSCs, adult stem cells 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 discover novel CSC surface markers, since the antigens recognized by the MAbs is highly likely to be present on CSCs, but not on normal tissue cells, in adults.

detection of CSCs in future studies. Most of CSCs were isolated by using monoclonal or polyclonal antibodies. Recent studies reveal that examination of the general protein expression is not sufficient to distinguish 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 also be 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 challenging, since 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, since adult stem cells are very rare in mature tissues, isolating these cells from an adult tissue is challenging, and culture methods to expand up to their required numbers is another task. Furthermore, when surface markers on adult stem cells are utilized as therapeutic targets against CSCs, there may be a possibility to eliminate normal adult stem cells and impair the normal process of tissue regeneration. Contrary to adult stem cells, hESCs or hPSCs are relatively easy to grow in culture. Among the 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 potential candidates as CSC markers, since surface markers of undifferentiated hESCs have oncofetal characteristics and are rarely expressed on normal tissue cells (Table 1 and Fig. 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 types of hESC surface markers deserve to be studied as potential CSC surface markers. Thus, finding novel surface markers on undifferentiated hESCs is 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.

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CONFLICTS OF INTEREST

The authors have no conflicting financial interests.

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