Review Article

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Recent update in identifying Cancer Stem Cells

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Abstract

Last three decades, significant revolutions have been observed in the field of Stem cell biology. Stem cells are quite complex. Several studies revealed their progression and transition of stem cells in various lineages has been effectively characterized in various systems. (CSCs) cancer stem cells remains in a variety of malignancies and they are the primary source of all tumors, which metastasis and relapse of the disease state. Various types of biologically distinct rare populations of "several tumor-initiating" cells have been reported in various cancers. CSCs, are dormant in nature and slow proliferating cells, under certain conditions, which can regenerate into a tumor. The present conventional therapies target only fast multiplying cells within the tumor, which tend to leave the quiescent or slow proliferative cancer stem cell population remains intact, which provides an opportunity to further reinitiate the tumor under favourable conditions. Moreover the current drugs are major failure in eradicating the proliferation of CSCs. Therefore developing new age therapeutics against CSCs is novel strategy, however there are limitations in Identifying CSCs. The Present short review highlights the importance of markers in identifying CSCs different types of cancers.

Keywords: Cancer, cancer stem cells, cell surface markers, therapeutic targets, CD cells,

Introduction

Studies related to stem cells are demonstrated that they are quite complex with their unique characterization, for the past 3-4 decades [1,2]. Studies revealed the progression and transition of stem cells in various lineages has been effectively characterized in various systems. The basic characteristics of various stem cell systems, has been demonstrated with the specific stem-cell properties. Similarly some cells exhibit same properties in human cancer are termed as cancer stem cells [1,3,4].

In general stem cells have unique property of self-renewal process to maintain themselves and when required they have capability to undergo differentiation into specialized cells [5]. The stem cells in most human tissues will undergo this process very rapidly and continuously in a systemic manner to maintain tissue homeostasis under controlled physiological conditions, and as well as to maintain balance between differentiation versus self-renewal [6]. Coming to the cancer cells, they will not follow the normal process of cell division, and reproduce uncontrollably and colonize in other places. The abnormal division of cells forms in to tumors or neoplasms, further categorised into either as benign or malignant. Invasiveness is the basic property of cancer cells, which allows them to enter into the blood or lymphatic vessels and metastases, to other places [7]. In tumors, majority of cells have limited self-renewal ability and are highly non-tumorigenic, however a fraction of subpopulation cells within the tumor mass have the ability of self-renewal capacity, which can further give rise to heterogeneous population of cancer cells [8].

To explain this two theories were proposed, and tried to solve this puzzle. The first theory or stochastic theory states that total cancer cells in the tumor are equally malignant but only few clones will possess favourable biological properties that will make them to grow upon transplantation. Whereas second theory proposes that tumors are hierarchical similar to normal tissues, only few set of subpopulation cells at the apex of that hierarchy will have the distinctive biological properties essential for tumor initiation. The importance of stem cells is recognized now in many solid tissue cancers. Irving L. Weissman, is the first person to coin this cells as, "Cancer stem cells", and stating that these cells arises from the malignant conversion of adult stem cells [4]. These cancer stem cells are the primary source of all tumors, which metastasis and relapse of the disease state. Various types of biologically distinct rare populations of "several tumor-initiating" cells have been reported in various cancers that include brain, breast, leukaemia etc [7].

Last three decades has seen significant revolution in stem cell biology. Recent advances in stem have contributed novel insights into field of cancer biology. Normal stem cells exhibit similar properties with that of cancer stem cells, most particularly the trait of self-renewal. This has laid foundation to the CSC theory, which states that a faction of subset population in tumor has the capacity to regenerate to full pledged tumor by producing all type cells.

By adding the tag of a 'stem cell' to these cells, are named as Cancer stem cells(CSCs), which are dormant and slow proliferating cells, under certain conditions, which can regenerate into a tumor. This could be the one valid reason why the present conventional therapies fail in most cancers. Moreover these therapies target only fast multiplying cells within the tumor, which tend to leave the quiescent or slow proliferative cancer stem cell population remains intact, which provides an opportunity to further reinitiate the tumor under favourable conditions.

Moreover CSCs origin is still unclear and under debate [2,5,8,9]. Few researchers proposed that CSCs are obtained from tissues with malignant changes, reside in special niches, responsible of distant metastases and have the capacity to rebuild the tumor after an otherwise effective therapy. CSCs have been reported in various tumors that incudes breast, brain, prostate, lung, ovarian, and liver tissue [8]. They are resistant against present existing therapeutic treatment strategies like chemo therapy and radiation therapy. This could be attributed as it may be due to with an effective DNA repair mechanism, followed by low levels of reactive oxygen species, and as well as high expression of multidrug resistance proteins [3,5,10]. Thus, targeting CSCs with appropriate new age therapeutics might eradicate cancer [6]. To achieve this, suitable methods are needed for the identification and separation of CSCs [3].

CSCs Identification

CSCs represent a very small percentage in tumor population, identified by unique characteristics. In order to assess them, it's essential to separate them from the total tumor population with appropriate procedures. For instance, these CSCs were separated from total tumor population using cell specific markers or CDs. These cell specific markers or CDs will varies on the type of the tumors. Moreover it is important that all the CSCs from various tumor population will not express same markers while some non-CSCs will express these. CSCs origin is still unclear and under debate. Few researchers proposed that CSCs are obtained from tissues with malignant changes, reside in special niches, responsible of distant metastases and have the capacity to rebuild the tumor after an otherwise effective therapy. CSCs have been reported in various tumors that incudes breast, brain, prostate, lung, ovarian, and liver tissue [3,8,9]. They are resistant against present existing therapeutic treatment strategies like chemo therapy and radiation therapy. This could be attributed as it may be due to with an effective DNA repair mechanism, followed by low levels of reactive oxygen species , and as well as high expression of multidrug resistance proteins. Thus, targeting CSCs with appropriate new age therapeutics might eradicate cancer [9]. To achieve this, suitable methods are needed for the identification and separation of CSCs.

CSCs have unique features like, they can auto regenerate, effectively proliferate and differentiate into either symmetrical or asymmetrical cell divisions, along with their tumorigenic potential. Initial identification and characterization is an important early step in assessing CSCs. For this various procedures were followed. Assessment of specific cluster of differentiation (CD) surface markers, testing sphere formation ability in soft agar or serum-free medium, dye exclusion capacity based on over-expression of drug-efflux pumps like ATP binding cassette or transporters related to multidrug resistance, and as well as stem cell based specific metabolic activities of aldehyde dehydrogenase 1, along with gene expression analysis were employed in the characterization of CSCs [5,7,8,11]. However, the most reliable method to assess CSCs only through in *vivo* assay, where induction of tumorigenicity can been studied in animal models after transplantation [5]. The various list of potential surface markers have been listed in (Table 1), for effective identification and separation of CSCs.

Table1: The CSCs markers in various cancers

Cancer/tumor types	Stem cell markers	References
AML(Acute myeloid leukemia)	CD34+,CD38–,HLA-,DR–,CD71–,CD90–,CD117–,CD123+	[4,5,8,13]
Acute lymphoblastic leukemia	CD34 +, CD38-, CD19+	[3]
Brain cancer	CD133+,BCRP1+, A2B5+,SSEA-1+	[8,10,13,31]
Breast cancer	Bcrp1+, ALDH+, CD133+, CD176+, CD56+, CD16+, CD44+,CD24-,ESA+	[5,10-12,12]
Bone sarcoma	Stro1+,CD105+,CD44+	[6]
Colon cancer	CD133+, CD44+, CD166+, CD166+, EpCAM+ , ALDH-1+, CD24+, CD26+, CD29+, Msi-1+ and Wnt activity/ β-catenin, CXCR4+, CK20+, CEA+, LGR5+	[3,5,8,17,18]
Endometrial cancer	CD44+, CD133, Oct4, Sox2, and Nanog	[16]
Gastric cancer	CD24+,CD44+,CD54+, CXCR4+,EpCAM+, ALDH1+, CD90+, CD71+,CD133+, CD166+, LGR5+, Oct4, Sox2	[19–21]
Gall bladder cancer	CD44+, CD 133+	[3]
Hypopharyngeal carcinoma	CD 271+	[29]
Kidney Cancer/renal carcinoma	CD105+,CD133-, CD24-	[3,29]
Liver cancer	Oct3/4, CD133+, CD44+, CD90+, CD13+, EpCAM+, ESA+, CD49f +CD24+	[5,8,22]
Lung Cancer	CD133+, CD44+, ALDH+, ABCG2+, Notch; Wnt; Shh	[1,23]
Mutiple myeloma	CD138-/CD19+/ CD20+/CD27+/ ALDH1+	[25]
Metastatic melanoma	CD20+, CD 271+	[6,29]
Prostate cancer	CD44+, CD133+ , CD44+, α2β1high	[8]
Pancreatic cancer	CD133+, CD44+, EpCAM+,CD24+	[8]
Oral cancer/Head and Neck cancer	CD44+, ALDH+, YAP1+, BMI1+	[26,27]
Ovary cancer	CD133 CD117+, CD24, CD44	[3]
Osteosarcoma	CD 271+	[29]
Thyroid Cancer	CD 271+, SSEA-1+	[28,29]
Testicular carcinoma	TRA-1-60 + SSEA-4 +	[30]

The use of surface markers will vary depending upon the tumor type. The surface markers like CD133, CD44 and CD24 are widely and commonly used to detect CSCs from solid tissue [1]. In addition, few more markers will be used in combination based on origin and type of the tumor tissue. For instance, the phenotype epithelial specific antigen (ESA^+) in combination with $CD133^+EpCAM^+$ $CD44^+CD24^+$ was used in identifying pancreatic CSCs , whereas $ESA^+CD44^+CD24^{-/low}$ and $ALDH\text{-}1^{high}$ (aldehyde dehydrogenase-1)

was the phenotype combination used in identifying in breast CSCs. Apart from this, Bcrp1 (Breast cancer resistance protein 1), CD133, CD176, CD56 and CD16 were also to detect breast CSCs [5, 10-12].

Various combination of selective surface markers like $CD34^{+}CD38^{-}$ HLA-DR^{-}(Human Leukocyte Antigen–antigen D Related), $CD71^{-}$ CD90–$CD117^{-}$ and $CD123^{+}$were used to identify CSCs of AML (Acute Myeloid Leukemia), however the markers like CD47, CLL-1(C-type lectin-like molecule-1), CD96, TIM3 (T-cell immunoglobulin and mucin domain 3), CD32 and CD25, were commonly expressed on other Leukemia stem cells. In case of Acute Lymphoblastic Luekamia the following markers like CD 34^{+}, CD 38^{-}, and $CD19^{+}$ were employed [3,4, 13-15]. For assessing CSCs of Endometrial cancer, markers like CD44, CD133, Oct4, Sox2 (SRY (sex determining region Y)-box 2), and Nanog were proposed [16]. In case of Colon cancer CD133, and CD 44,is the most common markers widely used, however, for better outcome various combination of markers were employed to determine CSCs that include CD133, CD44, CD166, CD166, EpCAM (Epithelial cell adhesion molecule), ALDH-1, CD24, CD26, CD29, Msi-1(Musashi homolog 1),$CXCR4^{+}$(C-X-C motif chemokine receptor 4), $CK20^{+}$,CEA^{+}(Carcino embryonic antigen), $LGR5^{+}$ (Leucine-rich repeat-containing G-protein coupled receptor 5) and Wnt activity/ β-catenin(3,5,8,17,18) . In determining CSCs of gastric cancer the following markers were proposed in combination, that includes CD24/CD44, CD54/CD44, CXCR4, EpCAM /CD44, ALDH1, CD90, $CD71^{-}$ CD133, CD166, LGR5, Oct4 and Sox2(19–21). Similarly the combination of Oct3/4, $CD133^{+}$, $CD44^{+}$, $CD90^{+}$, $CD13^{+}$, $EpCAM^{+}$ ESA^{+} $CD49f^{+}$ $CD24^{+}$ was used to detect CSCs in liver cancer(5,8,22). To identify CSCs in Lung cancer, the proposed combination of markers majorly include CD133, CD44, ALDH and ABCG2 (ATP-binding cassette sub-family G member 2), Notch, Wnt and Shh [1,23].

The cell surface marker $CD133^{+}$ is widely used for identifying CSCs in brain cancer, but in combination with other markers like $CD133^{+}$, $BCRP1^{+}$, $A2B5^{+}$ and $SSEA\text{-}1^{+}$ (stage-specific embryonic antigen 1) demonstrated better understanding [4,8,10,24]. The Prostate cancer CSCs are majorly identified with CD133+, however the combination of $CD44^{+}$, $CD133^{+}$, and $\alpha_2\beta_1^{high}$ (Integrin sub units) yielded better results [8,13]. The CD surface markers like CD34 and CD38 are widely used in identifying the CSCs of haematological malignancies, whereas in case of Mutiple myeloma, the following combination of markers were frequently used for better output that incudesCD138-/ CD19+/CD20+/CD27+/ALDH1+ [25]. In Oral cancer (head and neck), the CSCs were determined with the following combination of markers that includes CD44+, ALDH+, YAP1+ (yes-associated protein 1), BMI1+ (B lymphoma Mo-MLV insertion region 1 homolog) [26, 27]. Using the proposed combination of markers like Stro1+CD105+CD44+ the CSCs were identified in Bone sarcoma [6].

The CD cell surface markers for identifying CSCs in thyroid cancers is quite complex. In few studies it has been shown that markers like CD 271 and SSEA-1 were used to detect CSCs in thyroid cancers, However further studies are required for their effective usage [28,29]. Similarly, the identification of CSCs in testicular carcinoma was proposed by using markers like TRA-1-60 + and SSEA-4 + [30]. Interestingly, the Gall bladder cancer CSCs were detected with the combination of markers like CD 44+ and CD 133+ [3]. The CSCs in the ovary cancers were identified with the following markers in combination that includes CD133 CD117+, CD24, and CD44 [3]. The CD cell surface markers like CD20+ is used in identifying CSCs in Metastatic melanoma, however in another study CD271 was also proposed [6,29]. The combination of markers like CD105, CD133-, and CD24- were used to detect CSCs in kidney cancer/renal carcinoma, whereas CD271 used in to identify CSCs in hypopharyngeal carcinoma, and osteosarcoma [3,6,29].

In conclusion, the above review highlights the importance of potential markers for CSCs, however there are few limitations for identifying CSCs in various cancers. Moreover the present detecting methods has its own advantages and disadvantages, highlighting the significance of combinatorial approaches for the effective validation of CSCs in various types of cancers.

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