Very Small Embryonic-Like Stem Cells Transform Into Cancer Stem Cells and are Novel Candidates for Detecting/Monitoring Cancer by a Simple Blood Test

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Concise Review

Very Small Embryonic-Like Stem Cells Transform Into Cancer Stem Cells and are Novel Candidates for Detecting/Monitoring Cancer by a Simple Blood Test

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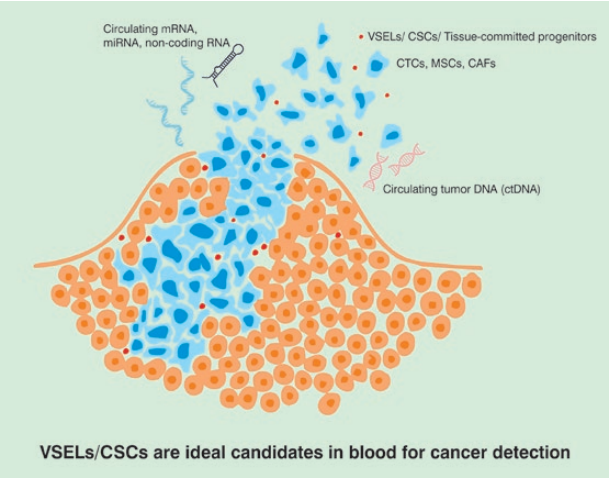
Abstract

Cancer continues to remain a "Black Box" as there is no consensus on how it initiates, progresses, metastasizes, or recurs. Many imponderables exist about whether somatic mutations initiate cancer, do cancer stem cells (CSCs) exist, and if yes, are they a result of de-differentiation or originate from tissue-resident stem cells; why do cancer cells express embryonic markers, and what leads to metastasis and recurrence. Currently, the detection of multiple solid cancers through liquid biopsy is based on circulating tumor cells (CTCs) or clusters, or circulating tumor DNA (ctDNA). However, quantity of starting material is usually adequate only when the tumor has grown beyond a certain size. We posit that pluripotent, endogenous, tissue-resident, very small embryonic-like stem cells (VSELs) that exist in small numbers in all adult tissues, exit from their quiescent state due to epigenetic changes in response to various insults and transform into CSCs to initiate cancer. VSELs and CSCs share properties like quiescence, pluripotency, self-renewal, immortality, plasticity, enrichment in side-population, mobilization, and resistance to oncotherapy. HrC test, developed by Epigeneres, offers the potential for early detection of cancer using a common set of VSEL/CSC specific bio-markers in peripheral blood. In addition, NGS studies on VSELs/CSCs/tissue-specific progenitors using the All Organ Biopsy (AOB) test provide exomic and transcriptomic information regarding impacted organ(s), cancer type/subtype, germline/somatic mutations, altered gene expressions, and dysregulated pathways. To conclude, HrC and AOB tests can confirm the absence of cancer and categorize the rest of subjects into low/moderate/high risk of cancer, and also monitor response to therapy, remission, and recurrence.

Key words: cancer; CSCs; VSELs; pluripotency; OCT-4; liquid biopsy; CTCs; ctDNA.

Graphical Abstract

VSELs/CSCs are ideal candidates in blood for cancer detection this statement here may not be needed as it is also mentioned in the bottom of figure.



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Significance Statement

Global disease burden and deaths due to cancer are on the rise. Early detection can help win the war against cancer. Tissue-resident, pluripotent, very small embryonic-like stem cells (VSELs), present in multiple tissues, transform into cancer stem cells (CSCs) due to various insults, initiate cancer and are also responsible for metastasis and recurrence. HrC Test has been developed, based on a published study using 1000 clinical samples, to detect cancers by examining VSELs/CSCs in circulation, while AOB Test is an NGS-based test that provides information regarding mutations and gene-expression changes in VSELs/CSCs/progenitors to better monitor/manage cancer patients. This potentially new diagnostic strategy is discussed and compared with currently available liquid-biopsy tests using CTCs, C-ETACs, and ctDNA.

Introduction

Cancer is a major public health problem and despite extensive research, overall fatalities due to cancer continue to rise globally.[1] The "War on Cancer" was declared about 5 decades ago, but, no "magic bullet" has been found so far to cure cancer.[2] There is no consensus on why cancer occurs in the first place, what are the underlying pathobiological mechanisms, and what causes metastasis and recurrence. The newly launched "Cancer Moonshot" initiative of US President Joe Biden aims to cut the death rate by at least 50% within the next 25 years and transform cancer into a chronic disease.[3] Early detection is the only way to achieve this since cancer can be treated effectively when it is a milder disease. Blood-based biosources such as circulating tumor cells (CTCs), cell clumps (circulating ensembles of tumor-associated cells C-ETACs), exosomes, cell-free nucleic acids (circulating tumor DNA ctDNA, long non-coding RNA, messenger RNA, and microRNA) offer an opportunity to detect cancer early and in a non-invasive manner. The present article discusses a potentially new strategy to detect cancer early in a liquid biopsy and monitor it based on circulating stem cells.

Circulating Tumor (CTCs) and Ensembles of Tumor-Associated (C-ETAC) Cells for Cancer Detection

Circulating Tumor Cells (CTCs), described 150 years ago, are sloughed off from the surface of primary tumors and offer promise for cancer detection, but are very rare (1 CTC in 10^6–10^7 leukocytes).[4,5] CTCs can be isolated physically based on size or by the immuno-adhesion method to achieve greater purity. The automated CellSearch® system (Menarini Silicon Biosystems Inc) is approved by the US Food and Drug Administration (US FDA) for quantification using EpCAM expression. Isolated CTCs are subjected to multiplex staining with immunofluorescent labeled monoclonal antibodies for epithelial cells (CK8/18/19), EpCAM, leukocytes (CD45), and a nuclear dye (DAPI) and visualized under a fluorescence microscope. The presence of 5 or more CTCs per 7.5 mL is a significant prognostic factor for poor survival of patients with breast cancer. However, despite these efforts, greater characterization of the genome, i.e., exome, transcriptome, and epigenome of CTCs is required for offering personalized treatments.[6] Unfortunately, this is beyond the scope of current technologies due to the rare nature of CTCs, lack of consensus on cell surface markers, heterogeneity, and short half-life (i.e., time taken to halve steady-state numbers) of CTCs (shed in lower numbers when cancer remains undisturbed, but in larger numbers when exposed to oncotherapy and/or surgery).

Circulating ensembles of tumor-associated cells (C-ETACs) have also been described in solid tumors. These are heterotypic clusters comprising tumor cells, immune cells, and fibroblasts, and their presence either singly or in clusters is suggestive of malignancy. The presence of C-ETACs in asymptomatic individuals is suggestive of a latent/yet undiagnosed malignancy and precedes the future diagnosis of cancer.[7] But the markers used to classify different types of cancers by immunofluorescence are not specific to any one cancer. Studying cancer stem cells (CSCs) may be a better option (discussed later) when compared with CTCs, C-ETACs, tumor-associated macrophages (TAM), and fibroblasts (CAFs).

Circulating Tumor DNA (ctDNA) for Cancer Detection

Circulating tumor DNA (ctDNA), a component of cell-free DNA (cfDNA) in blood, is another approach to cancer detection. Compared to CTCs which are responsible for metastatic spread, ctDNA gives specific information as a circulating nucleic acid biomarker,[8] for the presence/absence of specific alterations indicating therapy response. Many solid tumors and also CTCs in circulation shed pieces of DNA while undergoing apoptosis, but it accounts for only 0.1% of cfDNA in circulation. This DNA can be isolated and subjected to NGS which provides information regarding single nucleotide variants, insertion-deletion mutations, copy number variations, and tumor-specific patterns of methylation. However, the ctDNA may not allow for the detection of more than 65%-70% of cancers because patients with brain, kidney, and thyroid cancers as well as premalignant/early cancers release lower amounts of ctDNA in blood, as compared to patients with pancreatic, colorectal, ovarian, breast, gastroesophageal, and melanoma. ctDNA is released as a function of tumor size and stage of cancer, hence, it is relatively difficult to detect cancer "early" using this methodology. This approach is sensitive to late-stage cancers when the tumor has grown large and is shedding enough ctDNA in blood. ctDNA has a relatively short half-life of 30 minutes to 2 hours, hence undergoes rapid changes in blood stream in response to therapy, therefore, determination of mutations and methylation profile at steady-state levels might be challenging. The amount of blood required for studying ctDNA is large which ranges from 20 to 50 mL. Moreover, ctDNA comes from a heterogenous mix of cells, hence the interpretation of NGS data becomes difficult. Therefore, the industry is now focusing on single-cell DNA/RNA analysis rather than bulk analysis of heterogenous cells. Readers may refer to the published reviews on CTCs[9,10] and ctDNA[11,12] for recent advances in the field. A screening test should proactively be able to detect (compared to detection by accident during late stage as is the case at present) almost

all types of cancers at an early stage with equal sensitivity, in order to be effective.

Cancer Stem Cells (CSCs) and Cancer

Several unanswered questions exist in the field of cancer biology and it is essential to understand the underlying pathomechanisms to treat cancer effectively. Is stemness responsible for cancer initiation/metastasis, or do somatic mutations initiate cancer? Debate is still ongoing as to whether cancer is a cell-based, or a genetic molecular disease with cancer arising from a single cell due to accumulation of genetic mutations, or is it a tissue-based disease representing altered development. SMT (somatic mutations theory) vs. TOFT (tissue organized field theory) are being intensely deliberated to explain various aspects of cancer. A detailed discussion on this is beyond the scope of the present article but readers may refer to some excellent reviews for a greater understanding of this aspect.[13] The biggest evidence against SMT is that human embryonic (hES) and induced pluripotent stem (iPS) cells (devoid of any mutations) develop into teratomas or teratocarcinomas and can revert to normal when placed back in an embryonic environment.[14]

The stem cell theory of cancer initiation was proposed 4 decades ago and hypothesizes that CSCs drive metastasis and may be responsible for therapeutic resistance and recurrence. Readers may refer to reviews providing a historical perspective and an update on CSCs.[15-17] There is no clarity as to what are CSCs, what are their cell surface markers, and how to enrich them. There is an existing misperception in literature as to whether CSCs originate through dedifferentiation and reprogramming of differentiated progenitor cells to a pluripotent, stem-like state,[18] or originate from existing adult tissue-resident stem cells.

It was postulated that adult tissues harbor 2 populations of stem cells, including quiescent and actively dividing stem cells,[19,20] but it has proved difficult to detect the quiescent stem cells. As a result, it was suggested to identify stem cells based on their function rather than as definite entities.[21,22] CSCs (like normal stem cells) may not necessarily be rare and/or quiescent[22,23] and may arise through dedifferentiation and reprogramming of somatic cells.[24] Bhartiya's group has suggested that this debate is basically because of technical issues as the quiescent very small embryonic-like stem cells (VSELs) are inadvertently discarded while processing cells due to their small size and rare occurrence.[25,26] Since the initial controversy regarding VSELs[27] followed by various explanations,[28] robust methods are now available to enrich VSELs from blood as well as from multiple solid tissues.

Tissue-Resident VSELs and Cancer Initiation

Very small embryonic stem cells (VSELs) are endogenous, tissue-resident, pluripotent stem cells that exist in all adult tissues. A detailed description of VSELs is beyond the scope of this article and one may refer to published reviews.[29,30] VSELs are developmentally linked to primordial germ cells, serve as a backup pool to give rise to tissue-committed stem cells (TCSCs) "progenitors," and thereby play a crucial role in maintaining life-long homeostasis. VSELs are the true stem cells that exist in adult tissues while adult stem cells are indeed tissue-specific and lineage-restricted progenitors.[31] Differences between VSELs and adult stem cells and MSCs are provided in more detail in the Supplementary Section. The characteristic feature of stem cells (VSELs) is their ability to divide asymmetrically to produce a stem cell to self-renew, and a slightly bigger progenitor cell (Fig. 1). Asymmetrical cell division (ACD) produces 2 cells of different sizes with different fates due to epigenetic differences.[32] Distinct epigenetic changes occur during ACD in progenitor cells which become lineage-restricted and tissue-committed, compared to the pluripotent VSELs. This allows the 2 daughter cells to

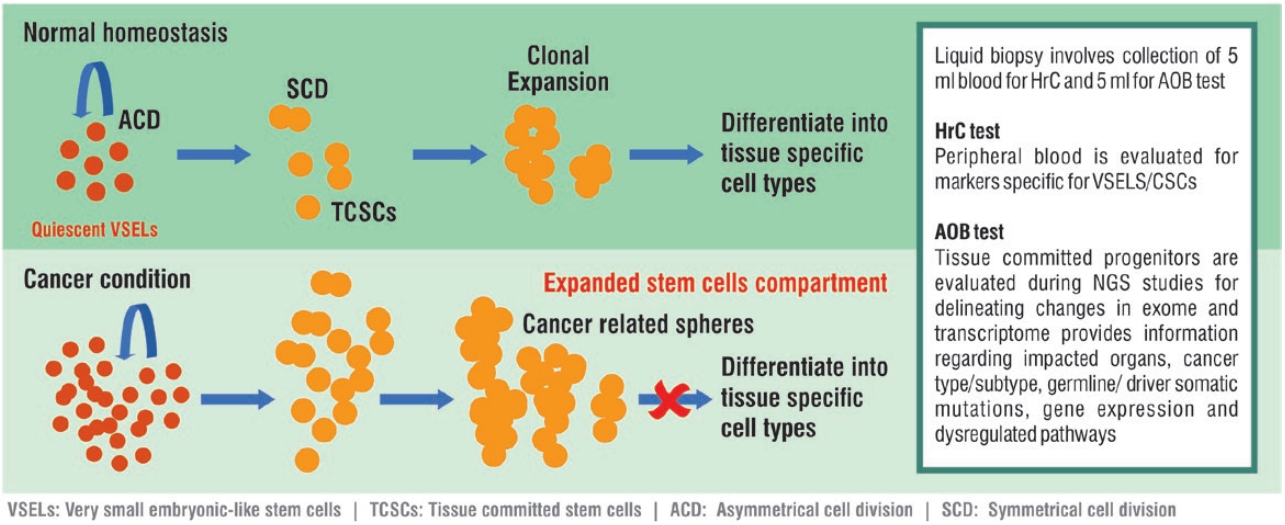

Figure 1. Role of VSELs in maintaining tissue homeostasis and how they get transformed into CSCs to initiate cancer. These cells in circulation offer a potential new approach for early detection of cancer in a liquid biopsy VSELs in adult tissues undergo rare ACD to give rise to tissue-committed stem cells 'progenitors' [like hematopoietic stem cells (HSCs) in bone marrow and spermatogonial stem cells (SSCs) in the testis]. These progenitors in turn undergo SCD and clonal expansion followed by differentiation into tissue-specific cell types. This understanding is derived based on studies done in mice including in the endometrium where these cell divisions and clonal expansion were also detected in vivo in tissue sections.[32,34] Various insults induce epigenetic changes and transform VSELs into CSCs which divide rapidly, form increased numbers of spheres and result in tumor formation while normal tissue differentiation gets blocked. [39] Increased numbers of VSELs/CSCs/TCSCs in peripheral blood are studied by HrC and AOB tests.

have unique transcriptomic profiles that lead to their specific cell identities. Progenitor cells further divide through symmetrical cell division (SCD) and clonal expansion, followed by differentiation to evolve into specialized, tissue-specific cell types with a finite life-span depending on where they reside. Actively dividing progenitors are responsible for daily tissue turnover for replacing damaged/dead tissue cells. VSELs get activated to restore homeostasis upon injury as reported after partial pancreatectomy[33] and under normal circumstances participate in normal tissue turnover including during regular remodeling of the endometrium during the estrus cycle in mice.[34] A balance between ACD of VSELs and SCD of progenitors is essential for maintaining tissue homeostasis; failure of which can lead to tumorigenesis.[35] To summarize, VSELs are ideal candidates for regenerative medicine and their altered epigenetic state and a defective niche possibly lead to cancer and age-related pathologies.[36]

Ratajczak's group was the first to postulate that VSELs are possibly the "embryonic remnants" described more than 150 years ago as part of the Embryonic Rest Hypothesis.[37,38] Evidence was recently generated in mice (Fig. 1) showing that VSELs exit their quiescent stage due to various endocrine insults, epigenetic changes, transform into CSCs, and undergo excessive self-renewal. The imbalance between ACD/SCD, excessive self-renewal, and impacted differentiation of VSELs, resulted in cancer[39] (Supplementary Fig. S1). CSCs exist as a distinct cell population in a tumor and are endowed with, just like VSELs, self-renewal, and multi-lineage differentiation capacity, and a characteristic resistance to cytotoxic agents.

Evidence suggesting the VSEL origin of CSCs is listed below:

- Both normal tissues as well as tumors are characterized by a hierarchical structure with stem, progenitor, specifically committed, and terminally differentiated cells. Like VSELs, being the most primitive stem cells in multiple tissues, CSCs also sit at top of the hierarchy and give rise to heterogenous lineages of tumor tissue including cancer cells, mesenchymal stromal cells, pericytes, cancer-associated fibroblasts, blood vessels, neurons, and nerve fibers.[37,38] Role of VSELs in providing tumor microenvironment is discussed in the Supplementary Section.
- Quiescence is the default state of both VSELs and CSCs. Both survive hypoxia. VSELs are quiescent in nature and survive oncotherapy similar to CSCs which results in recurrence.
- Seemingly immortal, both VSELs and CSCs exhibit the property of self-renewal, undergo asymmetrical cell divisions, and are resistant to apoptosis because of their quiescent nature.
- Both VSELs and CSCs get isolated as a side population implying that they are resistant to potential toxic chemicals including anti-cancer drugs which only kill cancer cells.
- Both VSELs and CSCs show the ability of clonal expansion and to form spheres.
- Both VSELs and CSCs express similar cell surface markers including LIN-ve, CD45-ve, and CD133+ve (humans) and SCA-1+ (mice).
- VSELs and CSCs express similar pluripotent, embryonic markers including OCT4, SOX2, NANOG, KLF4, MYC, CD166, CD133, ALDH1, ABCG2, CXCR4, and intracellular signaling pathways, including Wnt/β-catenin/NOTCH signaling.
- Both VSELs and CSCs have the ability to mobilize and migrate to distant organs.
- Both VSELs and CSCs exhibit plasticity and ability to expand in large numbers and differentiate into multiple lineages and promote angiogenesis.

Several poorly differentiated (malignant) cancers express pluripotent markers associated with embryonic stem cells.[40] Embryonic genes are expressed specifically by VSELs, and they exist interspersed with epithelial cells, in solid tissues including ovaries and endometrium. Thus, expression of pluripotent markers by various cancers is another evidence suggesting cancers arise due to selective expansion of VSELs and progenitor cells compartment as shown in Table 1. VSELs express estrogen (ERα, ERβ), progesterone (PR) and also gonadotropin (FSHR) receptors.[41-44] FSHR expression, reported in multiple cancers, is due to a selective expansion of VSELs that express FSHR.[45] Any dysfunction to basic stem cell biology during early development by various factors like endocrine disrupting chemicals, environmental pollutants, and other insults (eg, persistent infections, inflammation, obesity, autoimmune disease, or exposure to carcinogens) can result in various pathologies in adult life including cancers. Interestingly, diethylstilbestrol (DES), a non-mutagenic carcinogen, resulted in an increased risk of cancers in multiple reproductive organs of women in the early seventies and the effects were transgenerational. Similarly, neonatal exposure of mice pups to DES resulted in both testicular and uterine cancer in mice due to excessive self-renewal of VSELs and their blocked differentiation.[46-49] This was found associated with reduced P53 and PTEN, global hypomethylation (reduced expression of 5mC), loss of imprinting with increased IGF-1 expression, and dysregulated expression of Dnmts—all of which, resulted in the transition of VSELs to CSCs.[46-49]

This provides support to the postulate that cancer is a stem cell disease, and gets triggered by an imbalance between self-renewal/proliferation and differentiation of tissue-resident VSELs when exposed to various carcinogenic insults.

Metastasis, CTCs Versus CSCs

Metastases are multiple tumors that develop at a distance from the primary organ and are responsible for fatalities in majority of cancer patients. Patients more often do not die from the primary cancer (which can be removed surgically) but from metastatic tumors that spread to multiple organs. Mechanism underlying metastasis, by which cancer spreads from primary tumor to other sites and forms secondary tumors needs to be better understood. Metastasis is an inefficient process. Various steps like mobilization to distant places, successful seeding, invasion, and colonization at ectopic sites, eventually result in metastasis.[49,50]

In primary epithelial tumors, CTCs are epithelial cells that get sloughed off from the surface of primary tumors, undergo epithelial-mesenchymal transition (EMT), escape from the primary site into circulation, transit to secondary sites, and are believed to play a crucial role in metastasis and recurrence.[51] Although a primary tumor of 1 cm^3 size (roughly corresponding to 10^9 cancer cells) sheds one million cancer cells per day, but subsequent colonization is very limited due to incompatible distal sites. Less than 0.1% of disseminated cells successfully reach distal sites to trigger metastasis.

Table 1. Pluripotency marker OCT-4 expression in multiple cancers.

Leukemias	
	Aberrant expression and significance of OCT-4A transcription factor in leukemia cells https://doi.org/10.1016/j.bcmd.2014.07.014
	High expression of OCT4 is frequent and may cause undesirable treatment outcomes in patients with acute myeloid leukemia DOI: 10.1007/s13277-015-3731-5
	A three-gene signature might predict prognosis in patients with acute myeloid leukemia DOI: 10.1042/BSR20193808
Solid cancers	
Carcinomas	
Lung	Pluripotency transcription factors in lung cancer-a review DOI: 10.1007/s13277-015-4407-x Expression and clinical significance of Oct-4 and E-cad in non-small-cell lung cancer DOI: 10.3892/ol.2015.3856 Clinical significance of cancer stem cell markers in lung carcinoma DOI: 10.18388/abp.2020_5498 Clinicopathological and prognostic significance of Oct-4 expression in patients with non-small cell lung cancer: a systematic review and meta-analysis DOI: 10.21037/jtd.2016.06.01 Relationship of Oct-4 to malignant stage: a meta-analysis based on 502 positive/high Oct-4 cases and 522 negative/low case-free controls DOI: 10.18632/oncotarget.5737
Testis	Homeobox genes and cancer: new OCTaves for an old tune DOI: 10.1016/s1535-6108(03)00277-0 Protein Expression of PTTG-1, OCT-4, and KLF-4 in Seminoma: A Pilot Study DOI: 10.3389/fendo.2019.00619 Testicular cancer in mice: interplay between stem cells and endocrine insults DOI: 10.1186/s13287-022-02784-5 OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. DOI: https://doi.org/10.1097/00000478-200407000-00014
Ovary	Small putative NANOG, SOX2, and SSEA-4-positive stem cells resembling very small embryonic-like stem cells in sections of ovarian tissue in patients with ovarian cancer DOI: 10.1186/s13048-016-0221-3 An experimental model for ovarian cancer: propagation of ovarian cancer initiating cells and generation of ovarian cancer organoids DOI: 10.1186/s12885-022-10042-3 Cancer stem cell-related marker NANOG expression in ovarian serous tumors: a clinicopathological study of 159 cases. DOI: https://doi.org/10.1097/igc.0000000000001105
Colorectal	Apoptotic pathways and stemness in the colorectal epithelium and lamina propria mucosae during the human embryogenesis and carcinogenesis DOI: 10.1016/j.acthis.2016.08.004 Expression of Oct-4 is significantly associated with the development and prognosis of colorectal cancer DOI: 10.3892/ol.2015.3269 The Identifications and Clinical Implications of Cancer Stem Cells in Colorectal Cancer DOI: 10.1016/j.clcc.2017.01.011 CD133 expression predicts for non-response to chemotherapy in colorectal cancer DOI: 10.1038/modpathol.2009.181 The POU5F1 gene expression in colorectal cancer: a novel prognostic marker DOI: 10.1007/s00595-018-1644-9
Skin	Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues DOI: 10.1016/j.cell.2005.02.018 Complement C3a promotes proliferation, migration and stemness in cutaneous squamous cell carcinoma DOI: 10.1111/jcmm.13959
Glioma	Expression profile of embryonic stem cell-associated genes Oct4, Sox2 and Nanog in human gliomas DOI: 10.1111/j.1365-2559.2011.03993.xExpression and prognostic value of Oct-4 in astrocytic brain tumors. DOI: https://doi.org/10.1371%2Fjournal.pone.0169129
Endometrium	Stem cells in human endometrium and endometrial carcinoma. DOI: https://doi.org/10.1097/pgp.0b013e3182102754 OCT4, Ki-67 and VEGF as prognostic factors in endometrial carcinoma and their role in the differentiation between atypical endometrial hyperplasia and grade 1 endometrial carcinoma: an immunohistochemical study. DOI: 10.21608/JCBR.2021.52448.1101.
Pancreas	Resveratrol inhibits pancreatic cancer stem cell characteristics in human and KrasG12D transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition DOI: 10.1371/journal.pone.0016530

Table 1. Continued

Leukemias	
Glioma, hepatic, and lung cancer	Cancer stem-like cells can be induced through dedifferentiation under hypoxic conditions in glioma, hepatoma and lung cancer DOI: http://dx.doi.org/10.1038/cddiscovery.2016.105 Expression profile of embryonic stem cell-associated genes Oct4, Sox2 and Nanog in human gliomas. DOI: https://doi.org/10.1111/j.1365-2559.2011.03993.x
Oral cancer	Relevance of cancer initiating/stem cells in carcinogenesis and therapy resistance in oral cancer. DOI: https://doi.org/10.1016/j.oraloncology.2013.06.010
Gall bladder	CD133(+) gallbladder carcinoma cells exhibit self-renewal ability and tumorigenicity DOI: 10.3748/wjg.v17.i24.2965
Hepatocellular carcinoma	BORIS up-regulates OCT4 via histone methylation to promote cancer stem cell-like properties in human liver cancer cells. DOI: https://doi.org/10.1016/j.canlet.2017.06.017
Breast cancer	Post-translational modification of OCT4 in breast cancer tumorigenesis. DOI: https://doi.org/10.1038/s41418-018-0079-6 Oct4 suppresses IR-induced premature senescence in breast cancer cells through STAT3- and NF-κB-mediated IL-24 production. DOI: https://doi.org/10.3892/ijo.2018.4391. Reduced tumorigenicity and drug resistance through the downregulation of octamer-binding protein 4 and Nanog transcriptional factor expression in human breast stem cells. DOI: https://doi.org/10.3892/mmr.2014.2972
Prostate cancer	Oct-4 expression-maintained stem cell properties in prostate cancer-derived CD133+MDR1+ cells DOI: https://doi.org/10.4314/tjpr.v8i1.14706 The prognostic significance of OCT4 expression in patients with prostate cancer. DOI: https://doi.org/10.1016/j.humpath.2015.12.008
Cervical cancer	Clinical significance of OCT4 and SOX2 protein expression in cervical cancer. DOI: https://doi.org/10.1186/s12885-015-2015-1 Human papillomavirus E7 binds Oct4 and regulates its activity in HPV-associated cervical cancers. DOI: https://doi.org/10.1371/journal.ppat.1008468 Upregulation of stem cell markers ALDH1A1 and OCT4 as potential biomarkers for the early detection of cervical carcinoma DOI: 10.3892/ol.2018.9381
Renal cell carcinoma	Stem cell markers (OCT4 and CD133) and ANXA1 expression in RCC and correlation to histopathological features. DOI: 10.4103/EGJP.EGJP_40_20 Expressions of stem cell transcription factors Nanog and Oct4 in renal cell carcinoma tissues and clinical significance. DOI: 10.3109/21691401.2015.1105238
Sarcomas	
	Cytokine Induced Killer cells are effective against sarcoma cancer stem cells spared by chemotherapy and target therapy. https://doi.org/10.1080/2162402X.2018.1465161 Stimulation of Oct-4 activity by Ewing's sarcoma protein. DOI: 10.1634/stemcells.2004-0375. Critical role of the fibroblast growth factor signaling pathway in Ewing's sarcoma octamer-binding transcription factor 4-mediated cell proliferation and tumorigenesis DOI: https://doi.org/10.1111/febs.14946

CTCs neither express pluripotent markers nor steroid hormone receptors. They have a limited life-span and exhibit senescence, unlike metastatic tumors which show excessive growth. Rather than CTCs, CSCs (epigenetically altered VSELs) better explain tumor initiation, metastasis, and recurrence. Unlocking the biology of CSCs during tumor formation and metastasis is potentially the key to winning the war against cancer.

VSELs usually reside in the basal region of epithelial cells in solid tissues and are expected to be inadvertently released and get mobilized along with the CTCs/MSCs/CAFs produced by EMT of epithelial cells, and together explain the concept of seed and soil associated with metastasis. Seeds are considered to be CSCs while the soil is provided by mesenchymal stromal cells (MSCs) and cancer-associated fibroblasts (CAFs).[52] It was also reported in lung cancer that CSCs facilitate metastasis by bringing their own soil.[53–55] Breast cancer metastasis in the brain, liver, lung, or bone resembles the histo-architecture of the breast, suggesting CSCs to have pluripotent characteristics and properties like self-renewal and ability to differentiate into multiple lineage cells. Stromal cells play an important role by providing paracrine support in recapitulating phenotype of primary tumor by CSCs at the distant sites.

Potential New Strategy for Cancer Detection Based on Stem/Progenitor Cells in a Liquid Biopsy: HrC and AOB Tests

We have developed a proprietary method to enrich VSELs, CSCs (epigenetically transformed VSELs), and progenitors of impacted organs from peripheral blood. HrC test is based on a study published using 1000 clinical samples (500 normal control and 500 cancer samples).[56] The test enabled us to detect 25 types of cancers including solid, hematological, and sarcomas, with >99% accuracy. A

common set of cancer-related biomarkers including pluripotent, epigenetic, proliferation, and tumor suppressor genes were studied and a scale has been developed to classify subjects undergoing the test into cancer absent group otherwise there exists a low, modertae or high likelihood of cancer presence.

Absent:

An HrC score of <2 is indicative of the absence of cancer.

Low likelihood of cancer:

An HrC score between 2 and 6 suggests a low likelihood of cancer.

Moderate likelihood of cancer:

An HrC score between 6 and 10 suggests a moderate risk of developing cancer.

High likelihood of cancer:

An HrC score of >10 is indicative of cancer presence.

In addition, it is possible to perform NGS (exome and transcriptome) studies on the DNA/RNA extracted from VSELs/CSCs/progenitors as part of All Organ Biopsy (AOB) test. Compared to NGS on solid tissues (which provides specific information regarding that particular tissue), NGS studies on circulating stem/progenitors provide information about multiple impacted organs and also allow for detection of somatic driver mutations effectively. Besides information on the somatic mutations, AOB test provides relevant information regarding cancer types, primary/secondary impacted organs, mutations, altered gene expressions, and dysregulated pathways based on transcriptomic studies (Supplementary Fig. S2).

Several companies are present in the global market offering liquid-biopsy-based early detection (Supplementary Table S1). But understanding that cancers initiate from VSELs, and how they transform into CSCs that cause metastasis and recurrence, is a significant breakthrough in the field. Applying the HrC test (based on VSELs/CSCs/progenitors in circulation) is possibly a better method for early detection and effective management of high-risk subjects early on, when the disease is mild. This can help fight the war against cancer at the level of stem cells.

Conclusion and Future Directions

VSELs and progenitors from the impacted primary/secondary organs get mobilized into peripheral blood much earlier. CTCs, C-ETACs, and ctDNA get detected only in cancer subjects and a negative report does not strongly imply cancer is absent, since "absence of evidence is not always evidence of absence." Whereas VSELs exist in peripheral blood at a steady state in control, and are increased in cancer subjects. VSEL-specific markers detected as a part of HrC test correlate with the presence of cancer, while NGS based AOB test provides exomic and transcriptomic information regarding cancer type, primary/secondary impacted organs, mutations, expressions, and pathways. VSELs are transformed into CSCs and are the potential novel candidates to detect cancer through a blood test. The present review provides significant scope for further research to win the war against cancer! Newer therapeutic options to treat cancer will hopefully emerge. Milder protocols need to evolve to treat cancer when detected early as most existing protocols are aggressive and designed for late-stage disease.

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Conflict of Interest

D.B., N.S., S.D., P.K., Anish.T., and Ashish.T.: are employees of a startup company in India named Epigeneres Biotech Pvt., Ltd. Anish.T. and Ashish.T: are owners of Epigeneres Biotech Pvt., Ltd. Manuscript approved by Epigeneres Biotech Pvt Ltd, Mumbai for submission EPI/REV/04/ 2022.

Authors Contributions

D.B.: discussion on various aspects, conception and design, manuscript writing, collection and/or assembly of data, final approval of manuscript. N.S.: discussion on various aspects, manuscript writing, final approval of manuscript. S.D.: discussion on various aspects, manuscript writing, final approval of manuscript. P.K.: discussion on various aspects, manuscript writing, final approval of manuscript. As.T.: discussion on various aspects, financial support, administrative support, final approval of manuscript. An.T.: discussion on various aspects financial support, administrative support, final approval of manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

Supplementary Material

Supplementary material is available at *Stem Cells* online.

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