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Do PGCCs in Solid Tumors Appear Due to Treatment-related Stress or Clonal Expansion of CSCs that Survive Oncotherapy?

Deepa Bhartiya[1] · **Nripen Sharma**[1] · **Anish Tripathi**[1] · **Ashish Tripathi**[1,2]

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Abstract

Dedifferentiation of epithelial cells during epithelial-mesenchymal transition (EMT) results in circulating tumor cells (CTCs) that are mobilized singly or in clusters in association with blood cells and results in metastasis. However, lineage tracing studies have failed to delineate any role of EMT during metastasis. Research is also focused on polyploid giant cancer cells (PGCCs) in solid tumors which appear in response to oncotherapy-related stress for their role in metastasis. But how to explain PGCCs role in metastatic tumors in treatment-naïve patients? Studies done using mouse models and clinical samples suggest that cancer initiates due to dysfunctions of tissue-resident, pluripotent very small embryonic-like stem cells (VSELs). VSELs are the most primitive and pluripotent stem cells that exist at top of cellular hierarchy in multiple tissues. They are normally quiescent and undergo asymmetrical cell divisions to give rise to two cells of different sizes and fates including smaller cells to self-renew and bigger tissue-specific progenitors. Progenitors undergo symmetrical cell divisions and clonal expansion (rapid proliferation, endo-duplication with incomplete cytokinesis) to form giant cells that further breakdown and differentiate into tissue-specific cell types. Oncotherapy destroys actively dividing cells, but CSCs survive. We hypothesize that excessive self-renewal and clonal expansion of cancer stem cells (CSCs, dysfunctional VSELs) result in multinucleated giant cells (PGCCs) that accumulate as further differentiation into tissue-specific cell types is blocked in cancerous conditions. PGCCS are being reported by multiple groups whereas CSCs remain elusive due to small size and low abundance and actually contribute to both cancer initiation and metastasis.

Highlights

- VSELs, developmentally linked to primordial germ cells, are the most primitive, pluripotent, and quiescent (reversible cell cycle arrest) stem cells; undergo asymmetrical cell division (ACD) to give rise to tissue-specific progenitors that in turn undergo symmetrical division (SCD) and clonal expansion by undergoing rapid endo-reduplication with cytoplasmic connectivity to give rise to polyploid giant cell clusters. Extensive chromatin modeling occurs in progenitors, making them lineage-restricted and tissue-committed and they undergo further differentiation into tissue-specific cell types. Similar clusters termed PGCCs form in malignant solid tumors and CH/CHIP in hematopoietic tissue.
- Both chemo- and radiotherapy destroy actively dividing cancer cells while quiescent VSELs/CSCs survive. PGCCs appear after oncotherapy from the surviving CSCs as they undergo clonal expansion.
- PGCCs/CHIP accumulate because further differentiation is arrested in malignant cancers.
- Various extrinsic/intrinsic insults result in genomic instability, and somatic mutations occur mostly in the epigenetic modifiers and DNA repair genes during clonal expansion in PGCCs. Cancer initiates as a result of epigenetic changes and mutations are a consequence.

Keywords Metastasis · CTCs · PGCCs · VSELs · CSCs · Clonal expansion

✉ Deepa Bhartiya
deepa.bhartiya@epigeneres.com

[1] Epigeneres Biotech Pvt Ltd, Todi Mill Compound, Senapati Bapat Marg, Lower Parel, Mumbai 400013, India

[2] TZAR Labs, 23Ikigai Pte Ltd., 30 Cecil Street, #21-08 Prudential Tower, Singapore 049712, Singapore

Background

Cancer metastasis remains incurable despite the recent advanced cancer therapy strategies and results in >90% of cancer-related deaths. Unlike primary tumors which can be

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surgically removed and cured by localized radiotherapy, metastasis being a systemic disease, a lot of interest exists in screening, chemotherapy, targeted therapy, and immunotherapy. But despite various therapeutic advances, 5-year cancer survival rates have remained stagnant during the period 2000–2014 [1]. As a result, there is a lot of interest in delineating the underlying mechanisms leading to metastasis that could also lead to the development of better therapeutic options. Diverse views exist but most steps during metastasis remain poorly studied because of the absence of methods and models to study viable circulating tumor cells (CTCs) and characterize them [2].

The role of epithelial cells has been suggested in both origin and metastasis of solid tumors. These cells dedifferentiate during epithelial-mesenchymal transition (EMT) into mesenchymal cells get mobilized into circulation as CTCs. CTCs exist singly or in clusters and the clusters have 23–50-fold increased metastatic potential as they are more resistant to apoptosis [3]. These clusters could be homotypic (made of cancer cells only) or heterotypic (stromal or immune cells incorporated with cancer cells). Immune cells mostly of myeloid lineage are reported in these clusters and protect the mobilized cells from immune attack. Various stromal cells include fibroblasts, endothelial cells and platelets [4]. Multiple groups are working in this field and several lines of evidence have been published using tumor tissue and animal models. Recently Kuzedar et al. [5] reported a proof-of-concept study on breast cancer patients, where treating with digoxin (inhibitor of sodium and potassium transporter protein) resulted in an average reduction of 2.2 cells per CTC cluster which initially comprised around 4 cells [5, 6].

Another direction in which metastasis-related research has progressed is the crucial role being reported for the polyploid giant cancer cells (PGCCs) in solid tumors. PGCCs appear in response to therapy-induced stress like after chemotherapy and are suggested to have a fundamental role in metastasis and recurrence [7–10]. While CTCs clusters represent an aggregation of dedifferentiated epithelial cells and blood cells, PGCCs result from cell division abnormalities, may be polyploid, mononucleated or multinucleated giant cells, and harbor genetic aberrations. It has been suggested that CTC clusters may harbor a small percentage of PGCCs but essentially the CTC clusters are the products of life cycle of PGCCs. The presumption that differentiated epithelial cells exhibit plasticity and undergo EMT or partial EMT into CTCs that mobilize and metastasize at distant sites by undergoing reverse MET remains uncertain especially since lineage tracing studies have failed to delineate any role of EMT during metastasis [11, 12]. Also, if PGCCs appear in response to stress, what is their role in cancer origin, and how do we explain aggressive, metastatic tumors in untreated patients who are not exposed to any therapy-related stress?

More than 90% of solid tumors originate from the epithelial cells but these cells have a limited life span and undergo regular turnover from tissue-resident stem cells. Although the identity of these stem cells lacks consensus, a possible role of pluripotent, very small embryonic-like stem cells (VSELs) that exist at the top of the hierarchy in multiple tissues has been reported by several groups [13]. Indeed, VSELs have the potential to win the three-front war on tissue damage, cancer, and aging [14]. A possible role of VSELs in cancer origin was suggested by Ratajczak's group initially [15] and Virant-Klun's group has reported pluripotent VSELs in human ovarian cancer biopsies [16–18]. Our group recently provided evidence based on studies done in mice models that cancer possibly initiates due to epigenetic changes in tissue-resident, pluripotent VSELs and somatic mutations are a consequence of cancer [19]. Increased OCT-4 A expression (a specific marker for pluripotent cells) has been reported in the peripheral blood of patients with multiple types of cancers [20] and we have developed a pan-cancer liquid biopsy test for early prediction of cancer based on OCT-4 and other pluripotent markers [21]. This test based on mobilized OCT-4 positive stem cells enables early prediction of cancer compared to CTCs.

In the present article, we put forth the hypothesis that besides origin, VSELs are also responsible for cancer progression, metastasis, and possibly recurrence. PGCCs that are being reported by several groups in tumor tissues having a crucial role during metastasis are possibly an outcome of the clonal expansion of the CSCs that survive oncotherapy. However, being rare and of small size, CSCs remain elusive while bigger PGCCS easily get detected and reported. Also, we propose that both benign and malignant cancers develop due to dysfunctions of VSELs as an alternative to the published view of dualistic origin of human tumors.

A Brief Introduction to Very Small Embryonic-like Stem Cells (VSELs)

Ratajczak's group was the first to report pluripotent VSELs in adult tissues [22] and has recently published an updated review describing the contributions of >60 independent groups on different aspects of VSEL biology [13]. In brief, VSELs are the most primitive, pluripotent, and normally quiescent stem cells sitting at the top of the cellular hierarchy in multiple tissues and are developmentally linked to primordial germ cells. Under normal homeostatic

conditions, VSELs exist in the G0 stage of the cell cycle and undergo occasional asymmetrical cell divisions (ACD) to self-renew and give rise to progenitors of slightly bigger size and distinct fate. These progenitors undergo symmetrical cell divisions (SCD) and clonal expansion [Fig. 1] to form multinucleated, polyploid, giant cells [23]. These structures eventually break into single cells and further differentiate into tissue-specific cell types to maintain homeostasis under normal conditions. ACD is a unique type of cell division that offers immortality to the stem cells and helps maintain their genome and long telomeres [24]. The balance between the proliferation and differentiation of stem cells is crucial in maintaining homeostasis and is affected by age and upon exposure to extrinsic or intrinsic insults that result in various pathologies including cancer.

Clonal Expansion of Tissue-specific Progenitors Results in the Formation of Giant, Multinucleated Cells in Normal Tissues

The VSELs biology in adult tissues lacks global consensus as it has remained technically challenging to detect and study them because of their small size, presence in very few numbers, and quiescent nature [25]. Over more than a decade, corresponding author's group developed methods to understand VSELs biology using mouse models and also published evidence showing their role in the regular turnover of epithelial cells and participation during regenerate upon chronic injury [26]. Here we briefly review published data to show that tissue-specific progenitor cells produced by asymmetrical cell divisions of VSELs undergo symmetrical divisions and clonal expansion to form giant structures that eventually break down and differentiate into tissue-specific cell types. Thus, the formation of giant cells by clonal expansion of progenitor cells is a normal feature of adult tissues.

Pancreas

The best way to study the activity of 'quiescent' stem cells in any tissue is by inflicting chronic injury because the regular turnover of cells occurs in a very subtle manner and the involvement of stem cells remains unnoticed. The pancreas is an organ that is highly disputed for the presence of stem cells. Some groups suggest there are no stem cells in the pancreas [27, 28] and as a result, cancer initiates due to the dedifferentiation of adult somatic cells by a process termed paligenosis [29]. Others speculate that cell fusion [30] or tissue-resident stem cells [19] may have a role in cancer initiation. Bhartiya's group reported that VSELs are activated upon partial pancreatectomy to regenerate the damaged pancreas [31]. Later the group also reported the activation of the stem cells by partial pancreatectomy in diabetic mice (diabetes induced by streptozotocin treatment) leading to the formation of giant clusters (Fig. 2) [32]. Nuclear OCT-4 positive VSELs and the progenitors and giant cells with cytoplasmic OCT-4 were detected in large numbers. The

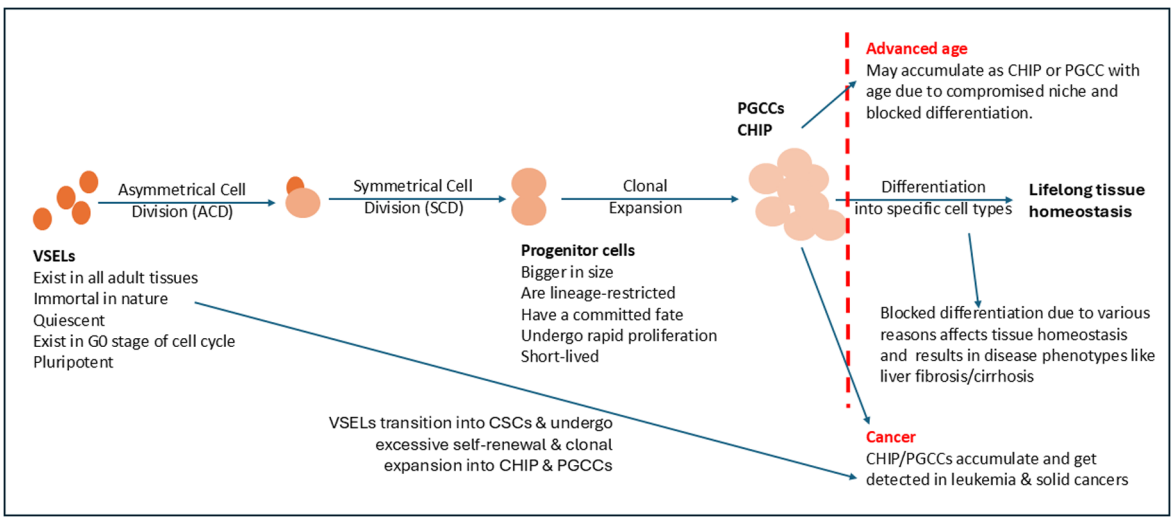

Fig. 1 Stem cell biology in adult tissues under normal and pathological states. Very small embryonic-like stem cells (VSELs) exist in adult tissues and their dysfunctions result in various pathologies including cancer [13, 19]. They undergo asymmetrical cell division (ACD) by which the smaller cell allows self-renewal while the bigger cell is a tissue-specific progenitor cell which further undergoes symmetrical cell divisions (SCD) followed by clonal expansion. The multinucleate structures formed due to rapid expansion (endoreduplication) and incomplete cytokinesis are being detected and termed PGCC or CH in solid cancer or leukemia respectively, and CHIP in tissues with no apparent disease state. Clonal expansion of stem cells is a normal feature of stem cells and occurs regularly in normal tissues, and aged tissues and are increased in numbers in various pathologies including cancer. It is crucial to understand that rather than targeting the clusters, cells that give rise to them are ideal targets for therapy. It will be ideal to normalize the altered epigenetic state of cancer stem cells and revert them to the quiescent state to cure/treat cancer

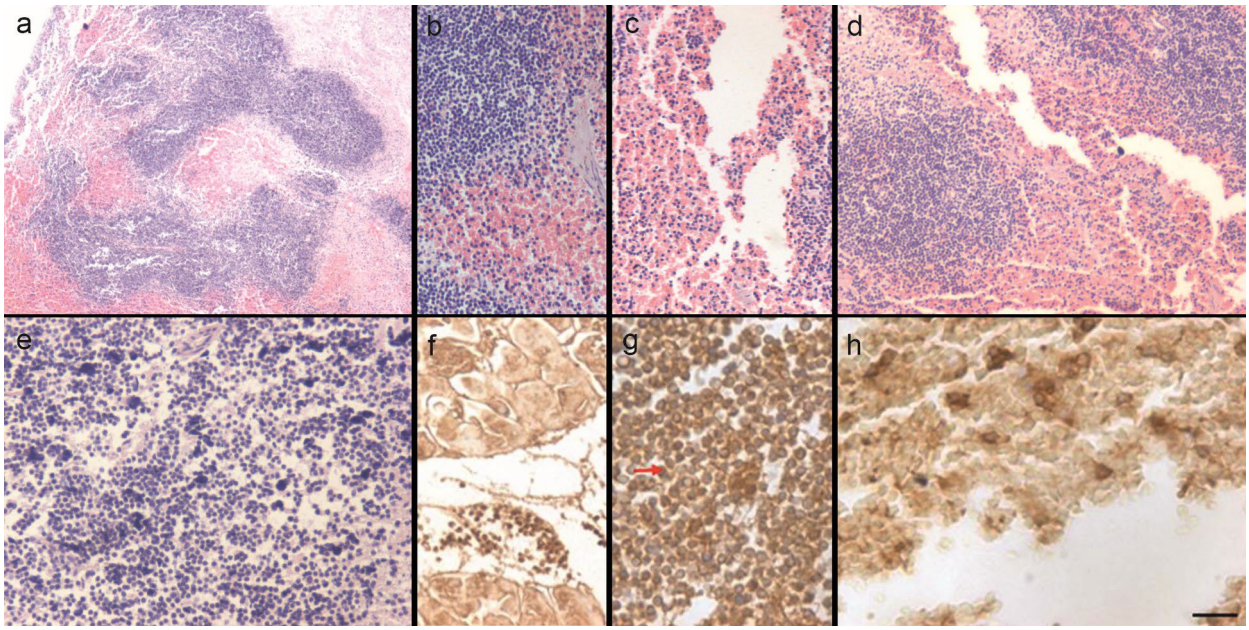

Fig. 2 Stem cells in adult mouse pancreas form PGCCs in response to chronic injury. Contrary to the existing belief that pancreas does not harbor stem cells and cancer initiates by dedifferentiation and 'Paligenosis', stem cells in the pancreas become distinctly visible upon inflicting chronic injury in mouse model. Here, diabetic mice were subjected to partial pancreatectomy and stem cells appear in large numbers at the cut surface (**a-c**). The stem cells appear dark blue, distinctly spherical and with high nucleo-cytoplasmic ratio. Clusters of stem cells representing PGCCs were seen at higher magnification (**d-e**). Two types of stem cells were seen including nuclear OCT-4 positive VSELs which were fewer in numbers and slightly bigger, more abundant pancreatic progenitors with cytoplasmic OCT-4. This work was published earlier [31, 32]

giant cells break down and further differentiate into acinar cells and islets.

VSELs are reported in several human tissues but not yet in normal human pancreas, however, indirect evidence for their presence exists based on studies done on pancreatic cancer. Herreros-Villanueva and colleagues [33] have reported the aberrant presence of key embryonic stem cell-specific markers like Oct-4, Nanog, and SOX-2 in cases of pancreatic ductal cell carcinoma. Lu and colleagues [34] showed that knockdown of Oct-4 and Nanog expression inhibits the stemness of pancreatic cancer cells. Wen and colleagues [35] reported expression of Oct-4 and Nanog during early stages of pancreatic carcinogenesis. Starzyńska and colleagues [36] reported intensified trafficking of $LIN^{-}/CD45^{-}/CD133^{+}$ VSELs and $CD45^{-}/CD105^{+}/STRO1^{+}$ mesenchymal cells in patients with pancreatic cancer. Similar giant cells as discussed in mice, will form and accumulate in pancreatic cancer due to excessive expansion of CSCs and since further normal differentiation is blocked. Several groups have published this view [37–39]. Other groups have also discussed ACD in cancer biology [37] and that PGCCs are formed by ACD [40, 41].

Ovaries

Germ cell nests or cysts are well-reported during early development in fetal ovaries. They comprise of inter-connected oogonia that later break down into individual oocytes and get surrounded by granulosa cells to assemble as primordial follicles [42, 43] and were also reported in adult naked mole-rat ovaries [44]. Bhartiya's group has worked on mouse ovaries for more than a decade to arrive at an understanding that these multicellular, giant germ cell nests form regularly in adult ovaries. The group initially reported the effects of treatment with an analog of follicle-stimulating hormone (pregnant mare serum gonadotropin, PMSG) on mouse ovaries [45]. PMSG treatment resulted in the formation of cohorts of primordial follicles on Day 2 which matured further by Day 7 after treatment (Fig. 3). Sriraman et al. [46] provided evidence that VSELs survived in the chemoablated (busulphan 10 mg/Kg daily for 4 days and cyclophosphamide 100 mg/kg on the first 2 days) mouse ovary and were stimulated by PMSG (5 IU, one month after chemotherapy) to clonally expand and form giant cells which are described as germ cell nests (GCN) or 'cysts' in ovaries. Later, Sharma and Bhartiya studied the cyclic changes in VSELs and the progenitors across the estrus cycle in mouse ovaries [47]. Increased numbers of GCN were observed in the estrus stage of the cycle in the ovaries and regularly differentiated into oocytes. Neonatal exposure to endocrine disruption resulted in polycystic ovaries in adult life and the stem cells in smears prepared upon enzymatic digestion of the ovary surface epithelial cells showed increased numbers of GCN that appeared growth

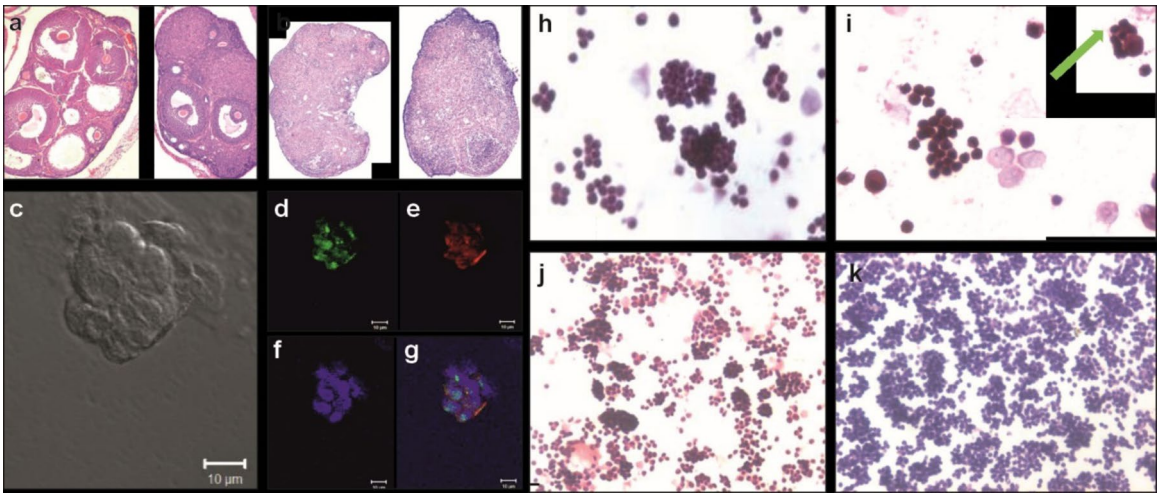

Fig. 3 PGCCs or cysts of germ cell nests in mouse ovary. (**a**) PMSG treatment resulted in the appearance of cohorts of primordial follicle below the ovarian surface that matured further and on D7 more cohorts were seen compared to untreated ovary [45]. (**b-c**) Sriraman et al. [46] studied the effects of chemoablation on mouse ovaries. As evident, follicular reserve was lost in chemoablated ovary, but PMSG treatment exerted a stimulatory effect on the OSE and proliferation and differentiation of stem cells into premeiotic germ cell clusters probably arising as a result of differentiation of the surviving VSELs on D6 during intact chemoablated ovaries culture. (**d-g**) Immunofluorescence expression of PCNA and OCT-4 in a PGCC with DAPI used as a counterstain. (h) Sharma and Bhartiya [47] showed the presence of germ cell nests in mouse ovary surface epithelial cell smears upon H&E staining. Their numbers were more during estrus. Under normal conditions, they participate in the differentiation of oocytes on a regular basis in adult ovary. (**i**) Neonatal exposure to endocrine disruption resulted in polycystic ovaries on Day 100 and the PGCCs were present with certain degree of blocked differentiation [48]. (**j**) PGCCS are seen in large numbers in aged mouse ovary surface epithelial cell smears upon H&E staining and (k) in cystic fluid collected from an aged mouse with bilateral ovarian cysts [49]. Results provide evidence that PGCCs form regularly in the mouse ovary, increase in numbers with age, and in various dysfunctions like PCOS and cancer

arrested [48]. Increased numbers of GCN were also detected in an aged, senescent ovary and in cystic fluid collected from an aged mouse with bilateral ovarian cysts [49].

Liu's group [50, 51] have reported PGCCs in ovarian cancer cell lines similar to primary ovarian cancer. PGCCs survive hypoxia and generate cancer cells by budding or bursting. PGCCs express embryonic markers (OCT-4, SOX2, NANOG, SSEA-1), differentiate into three germ layers, and form spheroids which upon transplantation developed into a wide variety of neoplasms. However, the group completely missed out on VSELs which are developmentally linked to primordial germ cells and could explain many of their findings. It is not that VSELs do not exist but remained elusive and were missed by the group as was discussed earlier [52]. Virant-Klun et al. [16, 17] have reported small putative NANOG, SOX2, and SSEA-4-positive stem cells resembling VSELs in ovarian tissue sections from patients with ovarian cancer. Later the same group reported the presence of CD133 + and DDX4 + VSEL-like stem cells in normal ovaries, ovarian cancer, and cultures of ovarian cancer ascites [18]. Findings from different groups, when combined, suggest that clonally expanded GCN or cysts are a normal feature in ovaries and possibly accumulate in cancer since their further differentiation is blocked.

Endometrium

The endometrium is one of the most plastic organs and undergoes regular remodeling including turnover of luminal and glandular epithelial cells across estrus cycles. Figure 4 shows the stem cells in a mouse uterus, present singly and undergo ACD, SCD and clonal expansion to form giant, multinucleated structures with cytoplasmic connectivity. Two distinct sizes of stem cells exist, including smaller VSELs and slightly bigger progenitors. These stem cells were further studied at a higher magnification and cytoplasmic connectivity between the cells was evident upon clonal expansion [23, 56, 57]. Singh et al. [26] showed that chronic injury inflicted in mouse uterine lumen with a needle resulted in increased numbers of OCT-4 positive stem and progenitor cells and underwent ACD, SCD, and clonal expansion. Further, the effects of neonatal exposure to endocrine disruption were studied on uterine stem cells. Increased numbers of VSELs and their compromised differentiation resulted in hyperplasia, infertility, lack of glands and cancer [58, 59]. Interestingly increased expression of pluripotent transcripts was observed in endometrial cancer including Oct-4 (> 100 folds), Oct-4 A (> 40 folds), Sox-2 & Nanog (> 60 folds), Sca-1 (> 35 folds), c-Kit (> 100 folds) [60].

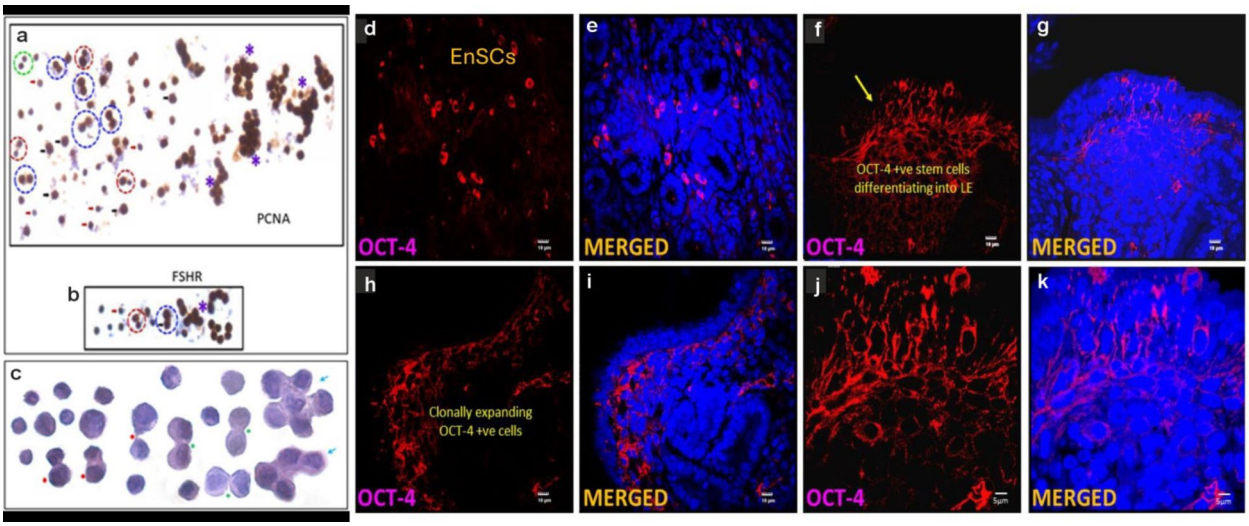

Fig. 4 Asymmetrical and symmetrical cell divisions and clonal expansion of stem cells in mouse endometrium in mouse uterus ACD (red broken circle), self-renewal of small VSELs (green broken circle), SCD (blue broken circle) and cell clusters representing clonal expansion. These are immuno-stained for (**a**) PCNA and (**b**) FSHR while (**c**) at high magnification [53]. (d-k) Clonal expansion of stem cells in mouse endometrial section. (**d-e**) OCT-4 positive cells increase in numbers in response to mechanical injury to the uterus [54]. (**f-g**) After 72 h of uterine injury, large numbers of tissue-specific progenitors EnSCs (endometrial stem cells) with cytoplasmic OCT-4 become evident (h-k) At higher magnification, EnSCs were observed to undergo symmetrical cell divisions and clonal expansion. Please note that these OCT-4 expressing stem/progenitor cells differentiate into epithelial cells and ensure widespread regeneration within 72 h of chronic injury. This is a good mouse model for solid tumors where tumor is initiated amongst the epithelial cells. Results suggest that epithelial tumors indeed initiate from the stem cells located amongst the epithelial cells because of altered expansion and differentiation of epithelial cells which eventually advance from hyperplasia-metaplasia to neoplasia. PGCCs will form in the process and get mobilized along with the stem cells to metastasize at distant sites. This data is already published [53–55]

Cardiac Tissue

Similar VSELs and clonally expanded cell clusters with cytoplasmic connectivity were also reported in mouse heart tissue (Fig. 5) [61]. The dysfunctions of tissue-resident stem cells in cardiac tissue most likely result in cardiomyopathies.

Bone Marrow

Bone marrow has been extensively studied for the presence of VSELs, in addition to HSCs by several groups [13, 62]. Ratajczak's group has shown that the VSELs survive due to their quiescent nature while the hematopoietic stem cells are destroyed when the mice are exposed to total body irradiation [63]. Bhartiya's group has reported asymmetrical and symmetrical cell divisions and clonal expansion (CH) of VSELs and HSCs in mouse bone marrow [64]. The mice were first treated with 5 fluorouracil (150 mg/Kg) that depleted the marrow of major cell types and enriched the stem cell compartment, later they were treated with FSH on Days 4 and 5 (5 IU per day), marrow cells were then isolated and used to make smears. ACD, SCD, and clonal hematopoiesis were evident (Fig. 6). Insert shows that the cells enriched by 5-FU in the bone marrow expressed OCT-4, smaller VSELs express nuclear OCT-4 while the bigger progenitors or HSCs express cytoplasmic OCT-4.

VSELs are also possibly implicated in leukemia as suggested by increased expression of pluripotent markers. Further studies need to be undertaken to investigate CHIP/CH in clinical samples of leukemia patients in different stages. Detection of CHIP in a person without any malignancy is suggestive of affected hematopoiesis and possibly represents early stage of leukemia. Recurrent AML-related mutations can accumulate within hematopoietic progenitors with age in healthy individuals, leading to clonal growth but without the development of leukemia. The most common CHIP-associated somatic mutations frequently occur in epigenetic modifiers including DNMT3 A, TET2, and ASXL-1. Mutations are also seen in genes related to DNA repair including tyrosine kinase gene JAK2, TP53, PPM1D, and SF3B1 [65, 66]. Increased incidence of CHIP is also reported in cardiovascular, kidney, and liver pathologies [67, 68]. We suggest all these are reflections of stem cell dysfunctions. Both CH in leukemia and CHIP in early stages reflect clonal expansion of the progenitors and become evident since further differentiation (hematopoiesis) is blocked.

Hematopoiesis gets affected with age with skewing towards myeloid lineage and reduced proliferation and differentiation of the lymphoid lineage [69]. This is directly associated with an increased incidence of developing myelodysplastic syndrome (MDS) due to the accumulation of defective cells in the marrow and the blood. It results

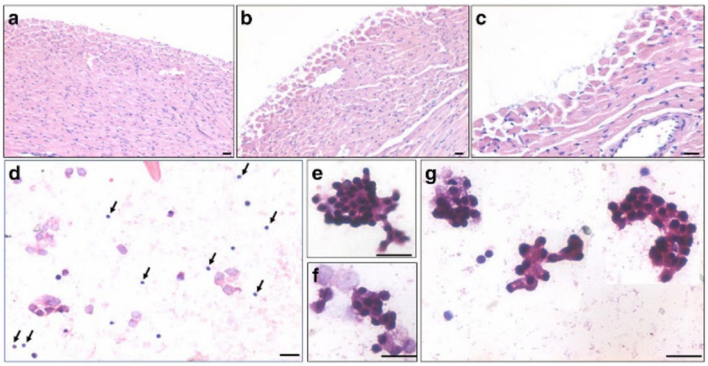

Fig. 5 Cardiac stem cells located in the outer pericardium of adult mouse heart form PGCCs. Hematoxylin and Eosin stained smear of cells obtained after partial enzymatic digestion of adult mouse heart tissue. Cells located on the heart surface were dislodged and collected as a single cell suspension and centrifuged at 1000 g otherwise these stem cells will not be observed as they remain buoyant when spun at 200–300 g. Darkly stained, small-sized, spherical stem cells with high nucleo-cytoplasmic ratio were observed interspersed with bigger cardiomyocytes with abundant pink cytoplasm and pale stained nuclei. a. H&E stained section of intact heart surface b–c. H&E stained section of heart showing damaged surface after enzymatic digestion. d–g. Two populations of stem cells including smaller, very small embryonic-like stem cells (VSELs, arrow) and slightly bigger cardiac stem cells (CSCs) were observed. They exist singly or as doublets and as small clusters 'cardiospheres' as a result of clonal expansion. Cytoplasmic connectivity is visible in the cell clusters and arises due to rapid proliferation and incomplete cytokinesis. The results suggest subtle stem cell activity (cell divisions and clonal expansion) and is published [61]

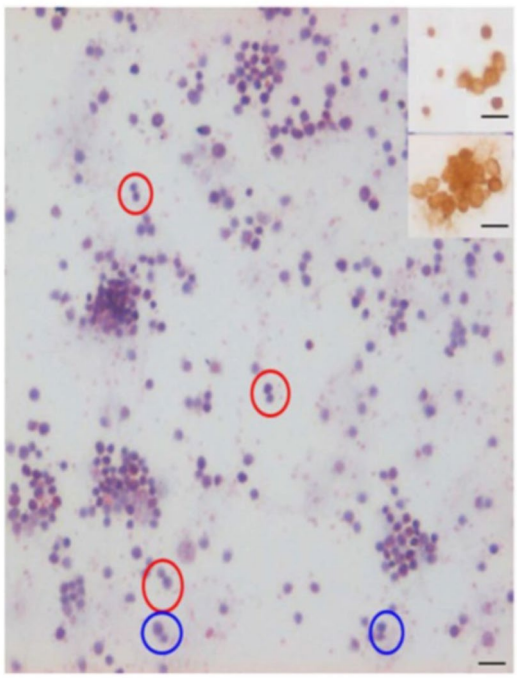

Fig. 6 Stem cell compartment and clonal hematopoiesis in the mouse bone marrow. H&E stained bone marrow stem cell smears prepared from chemoablated mice (5-flurouracil, 150 mg/Kg) after treatment on Days 4 & 5 with FSH (5 IU) and sacrificed on Day 6. Chemoablation resulted in the loss of major blood cell types and cell divisions of the surviving stem cells were evident including ACD (blue circle), SCD (red circle), and clonal expansion. Insert shows that the cells enriched by 5-FU in the bone marrow express OCT-4. Stem cells express FSHR and respond to FSH by undergoing ACD, SCD and clonal expansion [64]. Scale: 20 μm

in anemia (reduced RBCs), neutropenia (reduced WBCs) or thrombocytopenia (reduced platelets) that may progress to acute myeloid leukemia (AML) which is an aggressive hematological disorder [53]. OCT4, SOX-2 and NANOG are upregulated in acute myeloid cell lines and primary cells [54]. VSELs role in CML [55] and pediatric ALL [70] are also reported. Significantly higher expression of SOX2 and OCT3/4 antigens expression were reported in both ALL and AML at the time of diagnosis, which reduced in subjects with complete remission and the levels were elevated again at relapse [71]. These results suggest a role of VSELs in origin of leukemia and during relapse.

Altered Stem Cell Biology in Benign and Malignant Cancers

It remains unclear why CHIP is also linked to advanced age and pathologies affecting the cardiovascular system, kidneys, and liver [72, 73]. Giant, polyploid and multinucleated structures are also reported to form in response to acute injury to kidney to augment residual kidney function based on studies done in mouse models and also in human biopsies with kidney fibrosis after acute injury to kidney [74]. Similarly, the presence of somatic mutations in aged tissues [75] and in benign pathologies [76] besides in cancer tissues suggest that they are possibly associations and do not have a role in initiation of cancer. We suggest that formation of giant cells in the hematopoietic system and solid tissues in response to any kind of injury reflects clonal expansion of stem cells and accumulate somatic mutations as they exhibit

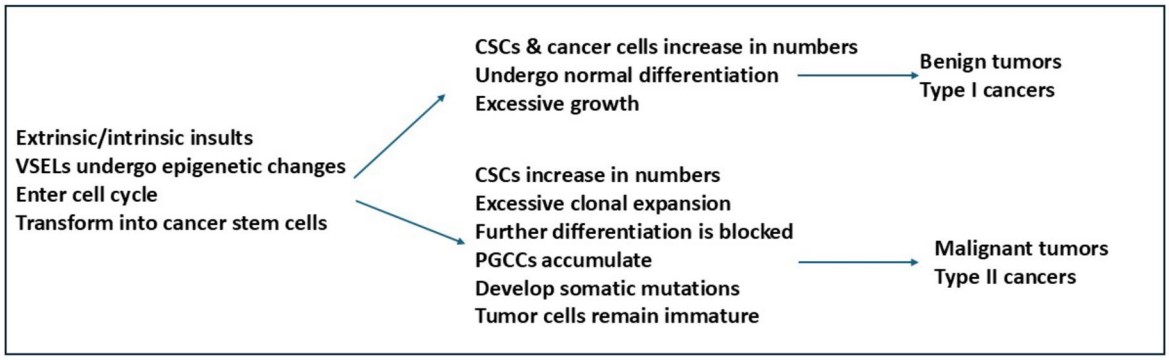

Fig. 7 Cancer stem cells (epigenetically altered VSELs) give rise to both Type I and Type II cancers. VSELs undergo epigenetic changes in response to various insults and enter cell cycle. They transform into cancer stem cells which undergo excessive self-renewal and clonal expansion. In Type I cancers (benign, no PGCCs, no somatic mutations) CSCs undergo normal differentiation into cells resembling normal cells. There is excessive growth in normal-looking cells in benign cancers. In case of Type II cancers (malignant, with PGCCs and somatic mutations) the CSCs expand in numbers and undergo clonal expansion but further differentiation into tissue cells is blocked. As a result, the PGCCs accumulate and develop somatic mutations. Cancer cells remain primitive and undifferentiated, express primordial germ cell-specific markers, and show similar characteristic features as discussed by Liu [77, 78]

genomic instability. We have earlier discussed that epigenetic changes in VSELs transform them into CSCs to initiate cancer while somatic mutations occur as a consequence of cancer when CSCs undergo clonal expansion [19].

According to the model of dualistic origin of cancer, Liu [77, 78] suggested that benign, Type I cancers initiate from stem cells arrested during early development from the blastomeres during embryogenesis while malignant, Type II cancers initiate as a result of dedifferentiation of mature somatic cells and are associated with PGCCs and somatic mutations. The group considers PGCCs as the potential source of CSCs or mother cells that can generate CSC-like progeny, contributing to tumor growth, resistance and metastasis [79]. Malignant tumors exhibit distinctive features like high nuclear grade, including hyperchromasia, increased N/C ratio, nuclear pleomorphism, and mononucleated or multinucleated PGCCs with poorly defined tissue architecture based on dedifferentiation of epithelial cells. The PGCCs have a role in metastasis and should be targeted to develop newer therapeutic options.

Cancer is a stem cell disease, and we have discussed the crucial role of pluripotent, tissue-resident VSELs. Normally quiescent VSELs undergo epigenetic changes in response to extrinsic/intrinsic insults and transform into CSCs, enter the cell cycle and undergo excessive turnover [19]. The balance between proliferation and differentiation is affected resulting in excessive growth. We propose that both benign and malignant cancers can be explained based on the dysfunctions of CSCs (epigenetically altered VSELs, Fig. 7) as an alternative to dualistic origin of cancer.

In benign cancers, CSCs (epigenetically altered VSELs) undergo excessive self-renewal and clonal expansion, but further differentiation of cancer cells remains unaffected. As a result, neither PGCCs accumulate, nor somatic mutations occur but excessive growth occurs with morphology similar to normal tissues. On the other hand, in malignant cancers, excessive increase and clonal expansion of CSCs lead to the accumulation of PGCCs/CH since further differentiation is arrested. These cells will develop somatic mutations while undergoing clonal expansion due to genomic instability. As a result of arrested differentiation, cancer cells remain immature and 'embryonic' in nature. VSELs are developmentally linked to primordial germ cells [13, 22], and this explains the expression of germ cell markers by metastasis initiating cells and cancer cells reported by several groups [80–83]. Moreover, distinctive features of malignant tumors like high nuclear grade, including hyperchromasia, increased N/C ratio, nuclear pleomorphism, and mononucleated or multinucleated PGCCs with poorly defined tissue architecture described based on dedifferentiation of epithelial cells by Liu [77, 78] are indeed distinctive and characteristic features of VSELs.

Discussion

As discussed above, dysfunctions of tissue-resident, pluripotent VSELs could result in the initiation of both benign and malignant cancers. The formation of multicellular giant cells is an integral feature of stem cell biology during regular turnover in normal tissues. VSELs survive radiotherapy and chemotherapy and get activated in response to various stimuli. We earlier reported that VSELs survive in chemoablated mouse ovaries and undergo clonal expansion upon treatment with PMSG [46]. Similarly, appearance of PGCCs in response to oncotherapy in tumors possibly represents clonal expansion of surviving CSCs (epigenetically altered VSELs). *PGCCs appear because of stress from the*

CSCs that survive oncotherapy. Being present in abundance and of bigger size, PGCCs are easily detected and reported by several groups while small-sized CSCs remain elusive. We recently discussed how misconceptions exist in the field of cancer biology as technological advances continue to confuse stem cell biology due to their inability to report cells of small size and rare occurrence [25].

More than 60 global groups have confirmed the presence of VSELs in different tissues since they were first reported in 2006 [22]. VSELS are pluripotent stem cells and their presence in adult tissues supports the Embryonic Rest Hypothesis put forth by Rudolf Virchow and Julius Cohnheim almost 150 years ago [15, 19, 84]. Our model based on VSELs having a role in cancer initiation (Fig. 1) is novel and challenges the concept of somatic mutation theory and dedifferentiation and reprogramming of epithelial cells as a source for cancer stem cells [85]. The scientific community is shifting away from gene-centric to cell-based mechanisms for cancer initiation and a recent article discussed the end of the genetic paradigm of cancer [86].

Quiescent VSELs are the real game changers in the field of cancer biology. Mechanisms underlying VSELs quiescence have been worked out [87] and their quiescent nature is further confirmed by the findings that they survive chemotherapy and radiotherapy. Ratajczak's group [63] reported that CD45- VSELs survived while CD45 +HSCs were lost in mouse bone marrow upon total body irradiation. VSELs not only survived but increased in numbers and mounted a regenerative response to restore homeostasis. Similarly, Bhartiya's group reported that VSELs survive chemotherapy in mouse ovaries and testes [46, 88, 89] and undergo clonal expansion in response to FSH treatment [46, 89]. Kurkure et al. [90] reported the presence of VSELs in otherwise azoospermic testicular biopsies of adult men who were affected by childhood cancer. Clinical details and type of oncotherapy provided to the seven subjects included in the study are provided in the original article [90]. Hematoxylin and Eosin-stained tubular sections confirmed azoospermia and cell smears obtained by enzymatic digestion of the testicular biopsy revealed the presence of very small, spherical VSELs with high nucleo-cytoplasmic ratio, in addition to Sertoli cells. Immuno-localization studies showed that VSELs were CD133+/CD45-/LIN-, expressed nuclear OCT-4, STELLA and cell surface SSEA-4. Pluripotent transcripts Oct-4 A, Nanog and Sox-2 were detected in azoospermic samples whereas germ cell markers including cytoplasmic Oct-4 and Boule were not detected. VSELs remain functional and have the potential to restore spermatogenesis upon transplantation of mesenchymal stromal cells for paracrine support as reported in multiple studies [88, 91] and are beyond the scope of the present discussion. What is intriguing to note is that these stem cells survived for >15–20 years in the human testes in a dormant/quiescent state and retain their functional potential. A recent study on leukemic blast cells in adult acute leukemia showed elevated levels of OCT-4 and SOX2 in patients with ALL and AML compared to controls, declined at remission and elevated again at relapse [71]. The reduction post therapy and then upregulation agrees with our view that stem cells expressing pluripotent markers survive and grow again leading to relapse.

The root cause for cancer origin, malignant transformation, invasion, metastasis, dormancy, recurrence and therapeutic resistance are CSCs that exist as VSELs in normal tissues. Trosko's group earlier discussed that instead of stating that embryonic, pluripotent genes are 're-expressed' during carcinogenesis, carcinogenesis prevents downregulation of OCT-4 which normally occurs as cells undergo differentiation [92]. They have reported OCT-4 expression in tumor cell lines from the pancreas and liver and also in HeLa and MCF-7 cell lines [92]. A recent study also reported OCT-4 isoforms in seven HNSCC (head and neck squamous cell carcinoma) cell lines. All 7 cell lines expressed nuclear OCT-4 while 4 of them also expressed cytoplasmic OCT-4 [93]. Lee et al. [94] have reported activation of OCT-4 by Wnt/β-catenin signaling in tumorigenic HNSCC cells similar to its role in maintaining the self-renewal of pluripotent stem cells. Similar molecular heterogeneity is also reported in liver cancer cell lines [95]. Established cancer cell lines, generally considered clonal, indeed remain highly genetically heterogeneous. The ability to collect PGCCs from cancer cell lines suggests that they may harbor VSELs which are developmentally linked to primordial germ cells, have high nucleo/cytoplasmic ratio, express OCT-4, survive in hypoxic conditions, and in G0 stage of cell cycle. VSELs are immortal in vivo and whether they have survived so many decades in cell lines needs to be explored further but expression of OCT-4 A strongly suggests their presence. Being pluripotent, CSCs/VSELs and PGCCs can differentiate into malignant cells, and stromal cells, undergo vascular mimicry, endothelial cells, and primitive blood cells with embryonic/fetal hemoglobin.

Rather than developing strategies to target PGCCs (which will invariably result in a recurrence from the surviving CSCs), therapeutic strategies need to be developed to revert the epigenetic state of CSCs and push them back to quiescence so that they start functioning normally. There is no need to destroy CSCs. We have published proof-of-concept studies in mouse models where various pathologies including cancer were reversed by treating with resveratrol (an epigenetic regulator) and by providing a healthy niche to the CSCs by transplanting mesenchymal stromal cells [96, 97].

There is a huge scope for further research on multiple fronts based on our hypothesis and basic scientists and oncologists with varied expertise need to interact to achieve

the cancer moonshot. The debate between the dedifferentiation of somatic cells versus the expansion in numbers of dysfunctional, tissue-resident stem cells leading to cancer needs to be settled.

Abbreviations

VSELs	Very small embryonic-like stem cells
CSCs	Cancer stem cells
ACD	Asymmetrical cell divisions
SCD	Symmetrical cell divisions
CH	Clonal hematopoiesis
CHIP	Clonal hematopoiesis of indeterminate potential
PGCCs	Polyploid giant cancer cells
MDS	Myeloid dysplastic syndrome
CTCs	Circulating tumor cells
ctDNA	Circulating tumor DNA

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