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Misconceptions Thrive in the Field of Cancer as Technological Advances Continue to Confuse Stem Cell Biology

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Abstract
Despite the huge thrust on targeted therapies, cancer survival rates have not improved and both cancer incidence and fatalities continue to rise globally. There is no consensus on how cancer initiates and two contrasting views were published in 2024 regarding cancer initiation. Based on the premise that no stem cells exist in tissues like liver, lungs, and pancreas but they are still affected by cancer; it was suggested that somatic cells dedifferentiate and undergo 'paligenosis' to initiate cancer. The second view discussed that tissue-resident, very small embryonic-like stem cells (VSELs) are vulnerable to extrinsic/intrinsic insults and their dysfunctions initiate cancer. The present article examines the underlying technical reasons that have led to these conflicting views. Scientists have struggled to detect quiescent cancer stem cells (CSCs) that survive chemotherapy, and radiotherapy and escape immunotherapy, cause recurrence and eventually therapeutic resistance leading to death. Lineage tracing studies fail to detect quiescent, acyclic stem cells and instead, the role of actively dividing LGR5+cells was highlighted for tumor initiation, growth, and metastasis. Similarly, technologies like flow cytometry, and single-cell RNAseq, widely used to comprehend cancer biology, provide insights into cell populations present in abundance. Our article reviews why VSELs/CSCs in the pancreas have remained elusive despite employing advanced technologies, and the critique can be generalized to multiple other organs. This understanding is crucial as it will help to develop better therapeutic strategies for cancer, offer early detection when cancer is a weak disease, and pave the path for prevention over treatment.

Keywords Cancer · Stem cells · Dedifferentiation · VSELs · CSCs · Pancreas

Introduction

Cancer is a disease in which some of the body cells grow out of control, spread to other parts (metastasis), and also show recurrence despite best personalized treatments. Treating oncologists struggle with issues like tumor heterogeneity, evolving nature of somatic mutations and therapeutic resistance. Cancer remains a major public health and economic problem; second leading cause of death and its burden is set to spiral. According to Globocan data [1], there were close to 22 million cancer cases in 2022 which are set to increase to 35 million by 2050. One in 5 people develop cancer in their lifetime, while one in 9 men and 12 women amongst those who contact the disease will die of it [1]. Despite global efforts on several fronts over a century, there is still no consensus on why and how this disease initiates in the first place. Two articles, published in the first quarter of 2024, discussed underlying mechanisms that lead to cancer initiation [2, 3]. They put forth contradicting views that are good for basic research, though consensus is desirable for winning the war against cancer in the Clinics. How can better management and a cure evolve if we do not even understand what goes wrong in various tissues that initiate cancer? Should strategies be developed to target mutations and to prevent dedifferentiation and reprogramming of somatic cells [2] or normalize the epigenetic state of the cancer stem cells and push them back to the quiescent state of tissue-resident stem cells [3]? This understanding is fundamental, and the present article attempts to delineate the underlying technical lapses made over more than two decades when

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the scientific community was actively engaged in studying endogenous stem cells in adult tissues that have led to the difference of opinion and are preventing a consensus in the present times on what cellular changes lead to cancer.

Alternative Explanations for Cancer Initiation Beyond the Gene-centric Perspective

One group opined that cancer occurs as an outcome of misbehavior (de-differentiation) of mature somatic cells harboring somatic mutations rather than stem cells [2]. The reasoning of this group was straightforward and based on published literature that organs like the liver, lungs, and pancreas develop cancer despite the lack of stem cells. They reasoned that cancer can initiate in the absence of stem cells from differentiated cells. But oval cells are well-reported in the lungs and have a role in the most aggressive type of metastatic lung cancer [4]. This newly emerging understanding of cancer initiation by the dedifferentiation of somatic cells has resulted in a Keystone symposium in 2019 followed by an eSymposium in 2020 and the coining of the term 'paligenosis' [5]. Paligenosis suggests that differentiated cells are 'plastic' and undergo dedifferentiation to an immature progenitor state during development, metaplasia, and tumorigenesis. According to this school of thought, differentiated cell architecture undergoes auto-degradation before it dedifferentiates and returns to the progenitor state. The group has suggested that during Stage I of paligenosis, mTORC1 is deactivated and autophagy gets activated, during Stage II embryonic markers get expressed like SOX9, TFF2, MUC6, CD44v while Stage III involves reactivation of mTORC1 and cell cycle entry. A few genes associated with paligenosis have been identified including IFRD1, Ddit4, etc. This concept is not new and several other groups have also suggested that dedifferentiation and reprogramming of somatic cells give rise to cancer stem cells in vivo [6–8], similar to the dedifferentiation and reprogramming of somatic cells in vitro into induced pluripotent stem (iPS) cells. In solid cancers, epithelial cells harboring somatic mutations undergo dedifferentiation while undergoing epithelial-mesenchymal transition (EMT) to attain stem cell-like features with self-renewal capabilities.

Our group discussed the role of tissue-resident, pluripotent, very small embryonic-like, stem cells (VSELs) in cancer initiation [3]. Various extrinsic/intrinsic environmental stresses affect the epigenetic state of normally quiescent VSELs, pushing them to enter the cell cycle and transform into cancer stem cells (CSCs). These CSCs initiate cancer, and somatic mutations occur consequently when the CSCs with genomic instability undergo rapid clonal expansion and their further differentiation is affected. This results in the rapid growth of cancer cells with somatic mutations which essentially remain embryonic and malignant [3]. This understanding is an outcome of extensive basic research in mouse models published earlier by Bhartiya's group [9]. CSCs (epigenetically altered VSELs) are possibly the root cause of cancer initiation, progression, metastasis, and recurrence. This view is in agreement with recent publications suggesting that cancer could be initiated by epigenetic modifications alone [10].

Several other views also exist to explain cancer initiation like the mitochondrial metabolic theory [11, 12] and dualistic 'life code' theory that unifies the human life cycle [13]. The presence of cancer-initiating cells has been studied in tumor tissues [14]. The evolutionary cancer theory was discussed recently [15] Besides initiation, no consensus exists even on how cancer metastasizes and recurs. Ratajczak's group was the first to suggest the role of VSELs in cancer [16]. VSELs are possibly the embryonic remnants that were considered to initiate cancer by Rudolf Virchow and Julius Cohnheim in the XIX century, as discussed in detail by Ratajczak's group [17].

Pluripotent VSELs: their Role in Maintaining Normal Homeostasis, Regeneration Upon Injury, Aging, and Initiating Cancer

VSELs were first reported by Ratajczak's group in 2006 and exist in multiple tissues including the liver, lungs, and pancreas [18, 19]. They have the potential to win the three-front war on tissue damage, cancer, and aging [20]. Different aspects of these stem cells have been studied over the years by more than 50 independent global groups [21, 22]. They have been extensively reported in umbilical cord blood and exist in all solid tissues (amongst the epithelial cells) and also in the hematopoietic system (bone marrow, peripheral blood) [22, 23]. VSELs became controversial in 2013 [24], the technical drawbacks were pointed out [25] and now after more than a decade of further research, robust protocols are available to isolate them from any solid tissue, and many more independent groups have confirmed their presence. Recently, Jarczak et al. [26] carried out scRNA-seq on VSELs sorted from the human cord blood, reported their transcriptional signatures, and showed that imprinted genes regulate their germ lineage origin. VSELs are the tissue-resident, most primitive, and pluripotent stem cells that sit at the top of the cellular hierarchy in all tissues. They are developmentally linked to primordial germ cells that get deposited and survive in all developing organs during early development and survive throughout life [22]. They are virtually immortal and exist in a 'reversible' quiescent state under normal conditions. Quiescence is a protective mechanism that helps VSELs evade various insults and prevents their exhaustion with

age. Figure 1 shows how they differ from the progenitors and adult stem cells that are well-described in tissues. Their functions get impacted with age due to a compromised somatic niche that regulates their functions.

VSELs lack a global consensus because despite being pluripotent, they do not exhibit few of the hallmark features described for human embryonic and induced pluripotent stem cells that are widely studied in vitro [27]. VSELs do not readily divide and expand in culture (although they differentiate into all three lineages and germ cells in vitro and *in vivo)*, neither form teratoma nor integrate into a developing embryo. This is because they remain in a state of 'reversible' quiescence under normal conditions due to their unique epigenetic state [28]. But these should not be the reasons to doubt their pluripotent state and potential. It needs to be stressed that epigenetic changes in VSELs due to various extrinsic/intrinsic insults result in cancer [3]. Multiple reviews were recently published discussing the progress made on pluripotent human embryonic and induced pluripotent stem cells since they were first reported 26 and 19 years ago respectively [29–31]. Despite the huge promise, these stem cells in vitro are yet to enter the Clinics. Besides genomic instability, risk of tumor formation, and immunological concerns, Yamanaka [31] highlighted significant heterogeneity among iPS clones and it is technologically challenging to select the best clone for each application. On the other hand, VSELs reported the same year as iPS cells, have exhibited real potential in clinics but largely remain unacknowledged and underexplored in mainstream cancer research.

Potential applications of VSELs for regenerative medicine were recently discussed [21]. They are activated upon chronic injury and participate in the regeneration in multiple models including mechanical injury to the uterine lining [32], partial pancreatectomy [33], CCl4-induced injury to the liver [34] and bleomycin-induced lung injury [35]. Importantly, the beneficial effects reported in a large number of studies upon transplanting mesenchymal cells or exosomes are possibly due to regeneration brought about by the resident VSELs in the presence of paracrine support provided by the transplanted cells [36]. Tripathi et al. [37] have successfully detected a wide variety of cancers in a double-blind 1000 samples study, by studying OCT-4A (marker specific for pluripotent state) positive cells (VSELs and CSCs) which get mobilized from the impacted organ into the peripheral blood for early diagnosis. The test is now available in Clinics to assess cancer risk in a liquid biopsy [38]. The presence of polyploid giant cancer cells (PGCCs) has garnered lots of interest, they appear in response to stress due to oncotherapy and are considered to result in metastasis [39–41]. However, being quiescent, CSCs survive oncotherapy and their clonal expansion could result in PGCC. To conclude, VSELs have entered the Clinics on multiple fronts and need to be acknowledged for their potential towards regenerative medicine and also by cancer

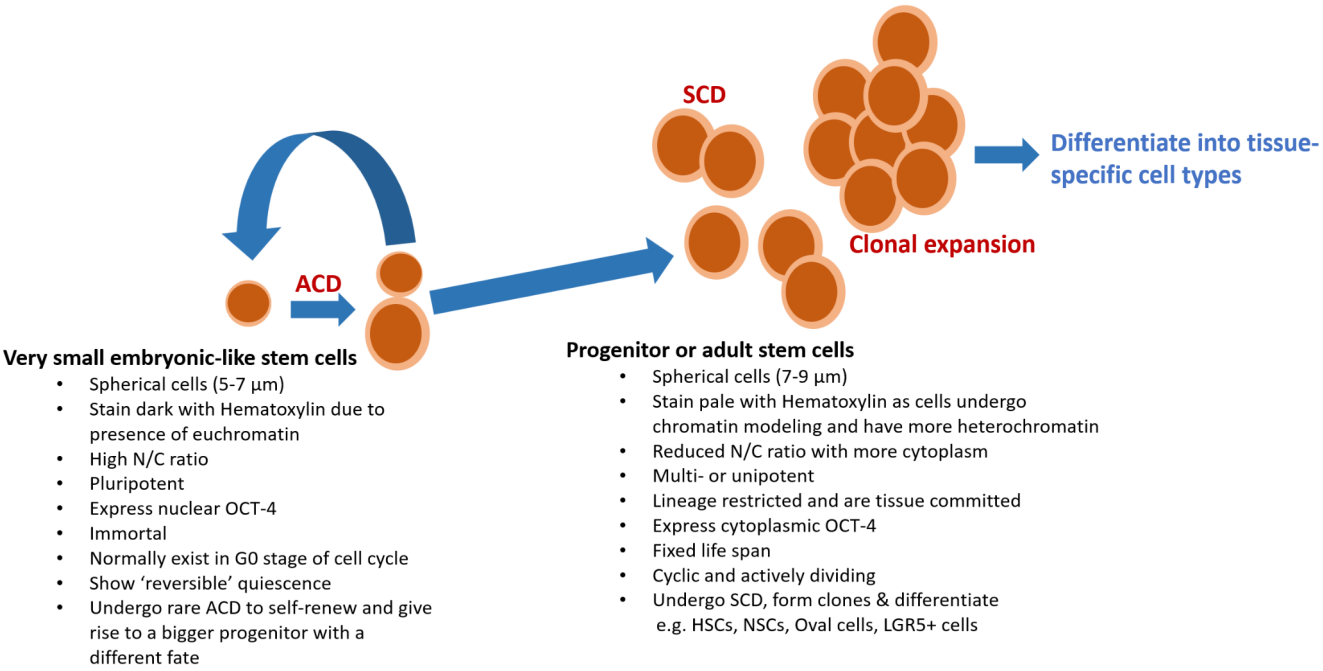

Fig. 1 VSELs and adult stem/progenitor cells comprise distinct subpopulations in adult tissues. VSELs are more primitive, sit at the top of cellular hierarchy, and undergo asymmetrical cell division to give rise to two cells of different sizes and fates while the adult stem/progenitor cells undergo symmetrical divisions and clonal expansion

biologists and oncologists for the crucial role in cancer initiation, progress, metastasis, and recurrence [3].

Personalized therapies including immunotherapies and targeting patient-specific somatic driver mutations have not been very helpful and despite extensive approvals, 5-years cancer survival rate has remained stagnant [42]. It becomes crucial to evolve novel therapeutic breakthroughs. A serious rethinking is required as to whether one should focus on developing strategies to prevent the dedifferentiation of epithelial cells or to manipulate dysfunctional VSELs to win the war against cancer. The elusive nature of VSELs because of their small size and being scarce, and because of their quiescent nature has resulted in several fallacies in the field of stem cells that have also crept into the field of cancer biology. Mills's group [2] based the concept of dedifferentiation or 'paligenosis' on the premise that the pancreas (and other organs) lacks stem cells while Bhartiya's group [3] discussed that VSELs exist in the pancreas (and other organs), participate in regeneration in response to chronic injury (discussed ahead) and their dysfunctions result in cancer.

Seemingly VSELs have remained elusive and unknowingly discarded in various studies because of their small size, rare occurrence, and quiescent nature. We suggest that the misinterpretations made more than two decades ago by multiple groups investigating the presence of stem cells in adult tissues have unfortunately snowballed and resulted in differences of opinion in the field of cancer biology existing in 2025. The scientific community is carried away by technological advances to publish something novel without appreciating the shortcomings of the techniques. The purpose of the present article is to discuss why such misconceptions and the "clash" between two opposite views have crept into the field of cancer biology in current times. Differences of opinion are always welcome in the field of basic research but when results get translated into the clinics, consensus becomes desirable. The present article is focused on delineating the underlying reasons that have led to the existing dilemma of what leads to cancer with a focus on pancreas biology studied using tools like lineage tracing studies, flow cytometry, and single-cell RNAseq. Similar reasoning can be applied to multiple other organs.

Lineage Tracing Studies Failed to Detect Quiescent VSELs in the Pancreas

Understanding the origin of pancreatic beta cells has profound applications not only for regenerative medicine to treat diabetes but also for better understanding of carcinogenesis. Do stem cells have a role in beta cells turnover or do the islets form by reduplication of pre-existing islets has been a subject of intense research and has been comprehensively reviewed [43]. The stem cells, if they exist, may be involved in regular turnover of islets, ensure regeneration after chronic injury (partial pancreatectomy), and may initiate cancer but in their absence, reduplication of existing islets may result in normal turnover of beta cells and cancer may initiate due to dedifferentiation and reprogramming of mature somatic cells. Leading groups and publications in high-impact factor journals addressed this in the early 2000s; surprisingly, the controversy is still not settled.

Melton's group at Harvard [44] developed a method for lineage tracing to determine the contribution of stem cells in the regeneration of pancreatic β cells. Transgenic mouse in which insulin promoter drives the expression of tamoxifen-dependent Cre recombinase16 (RIP–CreER) were used where the Cre–estrogen receptor (ER) fusion gene is expressed only in pancreatic β-cells. Tamoxifen injection resulted in a rapid and transient (about 48 h) nuclear translocation of the CreER protein, which permitted Cre-mediated recombination. This pulse of tamoxifen allowed HPAP (human placental alkaline phosphatase) expression in insulin-expressing cells present at the time of injection, as well as their progeny. They observed that all the islets stained with HPAP during both normal turnover and in response to partial pancreatectomy, leading them to conclude that islets renew by self-duplication of pre-existing islets and that stem cells have no role. Later they published a review article in Nature journal that there are no stem cells in pancreas [45]. Because there were no stem cells in the pancreas, research efforts were put into making beta cells in vitro from ES and iPS cells for regenerative medicine, but after 25 years of research, huge financial investment, basic research, and multiple publications, nothing has reached the Clinics yet [29–31] although diabetes was considered a low-hanging fruit for regenerative medicine. Bonner-Weir [46] discussed multiple pieces of evidence to support the neogenesis of islets. However, Magenheim et al. [47] with Douglas Melton as one of the co-authors in 2023 argued that there is insufficient evidence to support neogenesis and that lineage tracing experiments reported by Gibben et al. [48] had technical caveats. They concluded that existing evidence supports that homeostatic maintenance of pancreatic beta cells, and other pancreatic epithelial lineages, is primarily dependent on the proliferation of differentiated cells. Gribben et al. [49] responded and concluded that the topic remains controversial due to caveats associated with the lineage-tracing strategies and unambiguous validation remains to be achieved.

Similar to existing confusion in the field of regenerative medicine, confusion also prevails as to what causes pancreatic cancer. Based on multiple publications suggesting lack of stem cells in the adult pancreas in Nature and other leading journals, cancer biologists opine that differentiated cells

undergo paligenosis to initiate cancer. *However*, *we suggest that endogenous*, *tissue-resident VSELs have a role in regular turnover of islets*, *participate in regeneration upon partial pancreatectomy*, *could be targeted to regenerate the diabetic pancreas and also their dysfunctions possibly initiate cancer.* VSELs were initially reported in mouse pancreas by Ratajczak's group [17, 18, 22]. Bhartiya's group has also reported VSELs in mouse pancreas and also within the pancreatic islets [33, 40, 41, 50] (Figs. 2 and 3). VSELs were activated within 24 h of partial pancreatectomy resulting in the production of a large number of progenitors that differentiate into various cell types including β cells ensuring regeneration. As discussed above, VSELs are scarce, in a state of 'irreversible' quiescence in the G0 stage of the cell cycle under normal conditions, activated and enter cell cycle upon chronic injury, and return to quiescence once homeostasis is attained.

A re-look at the data from Melton's lab [35], keeping VSELs in mind, has an alternative explanation for why they failed to detect stem cells in the pancreas. Five injections of tamoxifen (4 mg, twice a week) were given over more than 15 days to ensure the nuclear translocation of Cre ER protein that allowed expression of HPAP. They are most probably and 'unknowingly' labeled VSELs-derived progenitors (which appear within 24 h of chronic insult) while injecting Tamoxifen over 15 days and thus all the β cells (even those that differentiated from the VSELs) stained positive for HPAP. Levine and Mercola [51] while commenting on their work had cautioned about the possibility that new beta cells could form from the stem cells after the pulse dose of Tamoxifen was administered.

Just as VSELs get activated in the pancreas upon partial-pancreatectomy [33, 50], VSELs are readily detected in mouse endometrium within 24 h of inflicting chronic mechanical injury [32] resulting in increased numbers of epithelial progenitors and regeneration within 72 h (Fig. 4). In both pancreas and endometrium, VSELs being scarce are not detected in normal tissue sections and function subtly to ensure regular turnover to maintain homeostasis but get easily detected under conditions of chronic injury.

The existing misperceptions in the fields of both regenerative medicine and cancer biology are due to technical caveats of various tools used to study quiescent stem cells in the pancreas. Lineage tracing studies will never be able to track non-cyclic quiescent VSELs nor delineate their role in both regenerative biology and cancer. The mistake, of failing to detect pluripotent stem cells in adult pancreas, that occurred two decades ago forms the premise for cancer initiation by paligenosis involving the dedifferentiation of epithelial cells in 2024 [2]. However, epithelial cells are differentiated cells with a limited lifespan and in a state of irreversible quiescence while VSELs are immortal in nature and transition

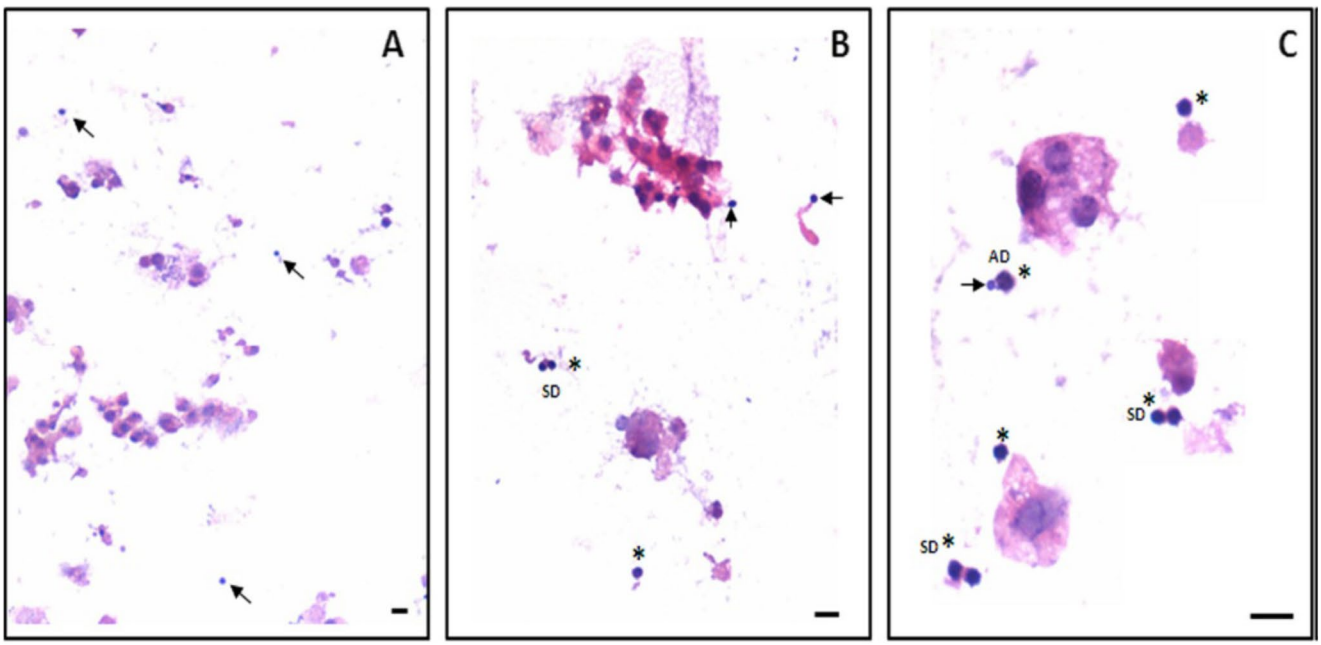

Fig. 2 VSELs in mouse pancreas. Haematoxylin and Eosin-stained smears of stem cells enriched from normal mouse pancreas at different magnifications 10X, 20X and 40X. Acinar cells are bigger cells with prominent pink cytoplasm (**A**). Please note the size difference between acinar and stem cells (arrow), because of which stem cells have eluded researchers to date. The stem cells have a distinct spherical shape, dark-stained nucleus, high nucleocytoplasmic ratio, and minimal cytoplasm. Somatic cells have relatively pale-stained nuclei. Two different sizes of stem cells can be appreciated at higher magnification (**B**, **C**). Smaller VSELs (arrow) divide and give rise to smaller cells to self-renew and bigger progenitors (asterisk) by asymmetric cell division (AD) and the progenitors further undergo symmetrical cell division (SD). Scale bar: 20 μm. This is published data [34] PMID: 25182166

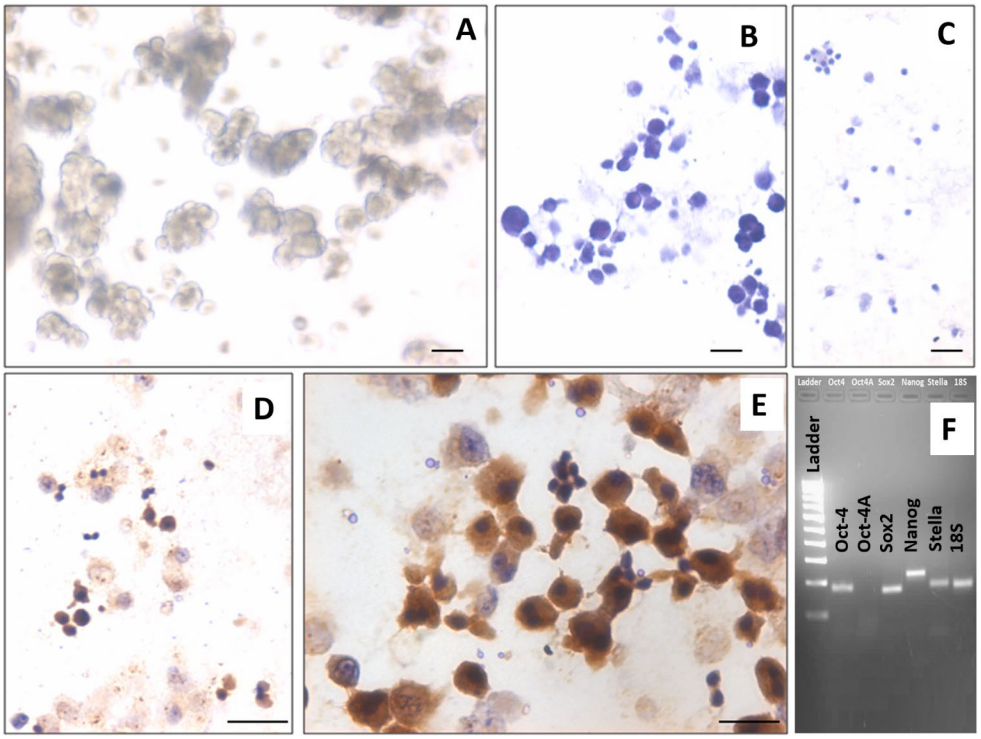

Fig. 3 VSELs in mouse pancreatic islets. Initially, the islets (large, variable-sized cell aggregates) were isolated from the adult pancreas (**A**) by following published protocols. Cell suspension obtained after trypsin digestion of islets comprised of bigger somatic cells (**B**) and small-sized, putative stem cells (**C**). Immuno-localization for OCT- 4 showed small-sized VSELs with nuclear OCT- 4 and slightly bigger-sized pancreatic stem cells (PSCs) with cytoplasmic OCT- 4 (**D**, **E**). Bigger cells are in different stages of differentiation and OCT- 4 expression is lost as the cells become more committed. Gel image shows expression of pluripotent markers in the RNA extracted from the islets (**F**). Note OCT- 4 A positive VSELs are visualized in cell smears but are not picked up by RT-PCR because they exist in a few numbers. This work is published data [35] PMID: 31705263

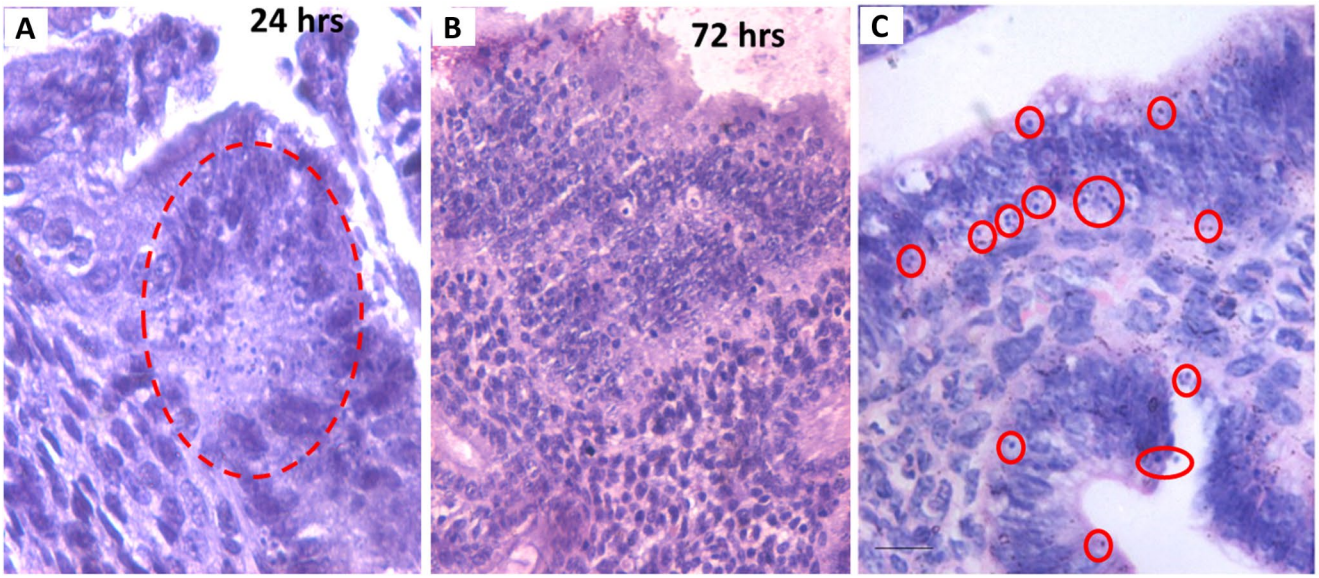

Fig. 4 VSELs exist amongst the epithelial cells in mouse endometrium. The small-sized VSELs (broken circle) become evident within 24 hours upon inflicting mechanical injury to the mouse uterine horn (**A**). They undergo differentiation into epithelial progenitors within 72 hours to restore tissue homeostasis (**B**). This data is published earlier in the supplementary section of PMID: 35123545 [37]. VSELs are seen in the H&E-stained endometrial section of an adult mouse neonatally exposed to endocrine disruption (**C**)

into CSCs to initiate cancer in response to various extrinsic/intrinsic insults [3, 43].

Lineage tracing has been used to study stem cells by multiple groups but the important prerequisite for this technology to work is the cyclic nature of the initial cell population [52]. A single cell gets marked, and information is obtained about the progeny of the founder cell, their location, and their differentiation status. Stem cells that divide infrequently, if labeled when actively cycling, will retain the label for several weeks and the dilution of label can be used to track their

progeny. VSELs have failed to be detected by lineage tracing studies because of their quiescent nature as discussed above. This has led to many misconceptions in the field of cancer biology. The ability to detect quiescent stem cells by lineage tracing studies is biologically impossible, a limitation of the technology and negative results do not justify suggesting that somatic cells dedifferentiate and reprogram into CSCs.

Almost 20 years ago, Hans Clevers' group studied stem cells by lineage tracing in intestinal epithelium, as it is one of the rapidly self-renewing organs in the body with cell turnover every 3–5 days. They detected Lgr5+ stem cells that were actively cycling and underwent clonal expansion to form organoids [53]. Having failed to detect quiescent stem cells through their studies, they published articles in high-impact journals including Nature Review Cancer suggesting that quiescent stem cells do not exist in adult tissues and called upon the scientific community to change the definition of stem cells [54, 55] and CSCs [56]. They did not realize the technical shortcomings of lineage tracing and this misinterpretation has now amplified on the global stage, resulting in recent reviews by Cho et al. [2] suggesting that rather than stem cells, cancer initiation involves dedifferentiation and reprogramming of somatic cells by undergoing paligenosis. But indeed, quiescent VSELs exist in all tissues, get epigenetically altered to initiate cancer, and could be targeted to win the war against cancer [3].

Hans Clevers group is a big proponent of use of organoids (actively dividing and clonally expanding structures capable of undergoing normal differentiation in vitro), reported in multiple organs including the stomach, liver, pancreas, prostate, and kidney, for modeling of human cancers and screening drugs for cancer therapy 'precision medicine' [57]. Organoids were named one of the top ten technologies of the year by *Science journal in 2013* and *s*ubsequently as Method of the Year by *Nature in* 2017. The most significant advantage of organoids is that they are human-derived and can simulate tumors in vitro. But the basic property of a stem cell is its quiescent state [58] while organoids represent actively and clonally dividing cells. Thus, the use of Lgr5+ cell-derived organoids for screening cancer drugs is possibly misleading. We need to screen and study the effects of various drugs on the CSCs (epigenetically altered VSELs) to achieve a cure for cancer [3].

A study by Fumagalli et al. [59] showed that although colorectal CSCs are $Lgr5^+$, $LGR5^+$ cells failed to seed metastatic disease. On xenografting human CRC tumor pieces into mouse model, $Lgr5^-$ cells formed distant metastasis while $Lgr5^+$ cells appeared later. In their graphical abstract, authors suggested that the $Lgr5^-$ cell progeny from $LGR5^+$ CSCs gets disseminated from the primary tumor to undergo metastatic colonization in distant organs. The findings were also discussed in Cancer Discovery and concluded that non-stem cells seed colorectal metastasis and gain stem cell properties over time (https://doi.org/10.1158/2159-8290.CD-RW2020-042.). Based on the results, the group wrongly concluded that CSCs are required for tumor growth but do not initiate metastasis. *This dilemma best explains the fact that actively dividing LGR5*$^+$*cells*, *identified by lineage tracing studies by them*, *are neither true stem cells nor have a role in cancer initiation or metastasis*. It remains an open question that needs to be answered, but it is likely that $Lgr5^-$ VSELs cause metastasis in the mouse model and give rise to the $LGR5^+$ cancer cells. This discussion suggests that defining stem cell markers based on lineage tracing experiments is misleading as it identifies actively dividing cell populations rather than quiescent stem cells. It is frustrating that although cancer is a 200-year-old disease and >90% of deaths occur due to metastasis [60], there is still no consensus on what causes metastasis and how to treat it. Multiple views exist as to which cells result in metastasis including the process of epithelial-mesenchymal transition resulting in circulating tumor cells [61, 62], polyploid giant cancer cells [39–41], and multiple factors facilitate metastasis including immune cells due to chronic stress [63]. It is amply clear that mitotic cells (including $LGR5^+$ cells) are not the ideal target, rather CSCs (with property of quiescence) need to be targeted for achieving a cure for cancer.

Pancreatic VSELs Remained Elusive during Flow Cytometry Studies

Xiao and colleagues [64] provided flow cytometry-based evidence to further support Melton's views that pancreas regeneration does not involve stem cells [44, 45]. The group used a tamoxifen-free technique wherein they employed a dual reporter system in which expression of Cre recombinase driven by the insulin promoter causes deletion of a red fluorescent reporter and simultaneously activates a green, fluorescent reporter. However, the inability to detect stem cells in their experiments was again because of technical and processing reasons. Inadvertent loss of VSELs (small in size with minimal cytoplasm) while processing cells for the experiments resulted in negative outcomes. We routinely obtain a 10-fold enrichment of VSELs by using robust protocols for preparing cell suspensions from various mouse tissues as confirmed by flow cytometry in mouse uterus, testis, and pancreas [33, 65, 66].

Single-Cell RNAseq (scRNAseq) Studies on Pancreatic Tissue

Another technical advance, scRNAseq was selected as the Nature Method of the Year 2013, breakthrough of the Year 2018 by Science journal, and spatially resolved transcriptomics was described as the Method of the Year 2020 by Nature. The technology has the power to provide genomic information at the cellular level compared to sequencing bulk cell populations in tissue that examines the average genome. It allows sequencing of the DNA and RNA of single cells and has the potential to transform many areas of biology and medicine. This technological advance is being used on a wide scale by various investigators to comprehend the biology of different tissue types. A lot of work has been done using scRNAseq to study how the pancreas responds to chronic injury, and also to gain insight into the underlying mechanisms leading to cancer. It has been suggested that any type of injury to the pancreas results in the transdifferentiation of acinar cells into ductal epithelium during 'acinar to ductal metaplasia' (ADM) by a process termed paligenosis and is a risk factor for cancer initiation. Tuft cells appear as a result of ADM and their ablation increases the risk of developing adenocarcinoma.

DelGiorno et al. [67] reported the presence of tuft cells in metaplasia and early-stage tumors along with the expression of SOX17 in human samples of pancreatic cancer. DelGiorno et al. [68] studied the tuft cells in multiple mice strains and reported cells co-expressing acinar (amylase) and ductal (cytokeratin) markers in response to an injury. Later her group [69] carried out scRNAseq to study the transcriptome of acinar cells in mouse models with pancreatic injury and compared it with that of human pancreatitis. They identified a distinct population of cells that expressed mucin and ductal markers, tuft cells, and enteroendocrine cells, similar to that reported in gastric metaplasia. They concluded that acinar cells possess plasticity and their dedifferentiation and reprogramming results in repair and also may lead to metaplasia. Another scRNAseq study was recently reported on pancreatic transcriptomes wherein they used a technical modification termed FixNCut by which tissue gets reversibly fixed with Dithiobis before dissociation and single cell preparation [70]. This allowed the transcriptome of acinar cells to be efficiently studied as normally these cells get degraded due to the digestive enzyme they produce. They reported pancreatic immune cells (neutrophils, macrophages, and DC cells), fibroblast, ductal, and endothelial cells and compiled a Pancreas Marker Atlas. Besides new insights into pancreas immunology and a bias towards type 2 immunity, they reported a set of genes specific to the ADM state.

Both the above-described scRNAseq studies failed to detect tissue-resident stem cells in the pancreas and rather data was gained on cell types that exist in abundance. This technology-related concern was discussed earlier also [71] that scRNAseq fails to detect rare cell populations and as a result, scientists are arriving at ambiguous interpretations and incorrect conclusions. Tissue-resident VSELs never get subjected to scRNAseq (as they get unknowingly discarded while processing cells), although they have a crucial role in both regular turnover and regeneration after chronic injury as discussed above [3, 32–35] rather than endoreduplication of preexisting islets or their appearance due to the plasticity of acinar cells after a chronic injury [44, 62, 69]. Cells co-expressing mucin and ductal markers reported by Ma et al. [70] could represent bipotent progenitors that arise from pluripotent VSELs in increased numbers in response to chronic injury rather than representing dedifferentiation of acinar cells.

Similar use of scRNAseq studies has resulted in a big confusion in the field of ovarian stem cells. A group from Karolinska Institute failed to detect stem cells in ovaries by scRNAseq [72–74] resulting in a debate [75, 76]. We have reported ovarian stem cells using very basic protocols [77, 78] and delineated their fascinating biology in mouse models that results in postnatal oogenesis, how they get affected by exposure to endocrine insults leading to various pathologies, and how their dysfunction results in age-related senescence and ovarian cancer [79–81]. An Ovarian Atlas is now made available describing various cell types including oocytes, granulosa cells, immune cells, endothelial cells, perivascular cells, and stromal cells [66]. A recent study published in Science by Jones et al. [81] also describes four major cell types in the human ovary (oocyte, granulosa, thecal, and stromal cells) and four immune cell subtypes by combined spatial and single-cell RNAseq studies. However, it is well known that the ovary is surrounded by a layer of simple squamous-to-cuboidal epithelial cells termed germinal epithelium, which were not detected in both studies [72, 81] besides the stem cells. Based on the results obtained from scRNAseq on ovarian tissue, it becomes evident that only cells present in abundance get preferentially detected by scRNAseq. Both epithelial cells and stem cells, being scarce, fail to get interrogated by scRNAseq- although they do exist and importantly > 90% of ovarian cancers initiate from VSELs residing in the surface epithelium [82].

Pancreatic Cancer Stem Cells Express Pluripotent Transcription Factors

Although the presence of stem cells in normal pancreas remains disputed, cancer stem cells are reported by several

groups in pancreatic cancer tissue and cell lines (Table 1). Interestingly pluripotent transcription factors OCT-4, SOX2 and NANOG show increased expression in pancreatic cancer and their knockdown and exposure to various therapeutic options have shown interesting results [83–92]. These results suggest that increased numbers of CSCs (epigenetically altered VSELs) are easily detected in cancer tissue whereas in normal pancreas, only cytoplasmic OCT- 4 is reported by a few groups since the VSELs remain scarce, although our group has reported VSELs in normal and diabetic mouse pancreas upon partial pancreatectomy [33, 50]. Increased expression of cells with nuclear OCT-4 in the hamster model of pancreatic cancer is strong evidence suggesting a role of VSELs in pancreatic cancer. Wen et al. [85] provided insightful data wherein they show that these markers are selectively increased in the pancreatic cancer cells compared to adjacent normal tissue, increased expression in cases with pancreatitis which is an early stage of cancer initiation. Also, in BOP induced pancreatic cancer in hamsters, they found that k-Ras mutation appeared later to increased expression of Oct-4, Sox2 and Nanog. These results support our view that cancer initiates due to an excessive increase

Table 1 Pancreatic cancer stem cells express embryonic markers

Tai et al. 2004 [83]	Using antibodies and PCR primers, human breast, liver, pancreas, kidney, mesenchyme and gastric stem cells, and tumor cell lines were studied for Oct4 expression. Tumor cells but not differentiated cells expressed Oct4.
Iki and Pour 2006 [84]	Pancreatic cancer was induced by *N* -nitrosobis(2-oxypropyl)amine) (BOP) Normal pancreas showed Oct4 expression only in islet cells in a diffuse cytoplasmic pattern. No nuclear staining was found in any cells. In 14 of the pancreatic cancers, nuclear staining was detected in many cells or in small foci. Nuclear staining was also identified in early intra-insular ductular and in Ca in situ lesions
Wen et al. 2010 [85]	Tissue microarray of human pancreatic carcinoma and adjacent noncancerous tissues were done and also BOP induced pancreatic cancer was studied in hamster pancreatic cancer model for Oct- 4, Nanog and Sox2. The presence of K-ras mutation with the time course of carcinogenesis in hamster model was also evaluated. Oct4 immuno-stained only in the cytoplasm of islet cells in the normal pancreas Strong nuclear Oct4 in the metaplastic ducts of tissue with chronic pancreatitis Oct4 was weak and heterogeneously stained in pancreatic cancer cells Sox2 and Nanog were prominently expressed in pancreatic cancer cells Oct4 expression preceding K-ras mutation in BOP induced pancreatic cancer in hamsters
Lu et al. 2013 [86]	Oct4 and Nanog showed increased expression in human pancreatic cancer tissues associated with worse prognosis. The pancreatic cancer stem cells (CD24 +CD44 +ESA +PCSCs) isolated from PANC- 1 cell line by flow cytometry showed high expressions of Oct4 and Nanog. Double knockdown of Oct4 and Nanog significantly reduced proliferation, migration, invasion, chemoresistance, and tumorigenesis of PCSCs in vitro and in vivo.
Wang et al. 2013 [87]	CSCs were studied in the side proportion (SP) cells in the human pancreatic cancer cell lines They possessed aggressive growth, invasion, migration and drug-resistance properties, compared to cells that were not in the SP. SP cells overexpressed stem cell markers CD133 and ALDH1, pluripotency maintaining factors Nanog, Sox2 and Oct4, oncogenic transcription factor c-Myc, signaling molecule Notch1, and drug-resistant gene ABCG2. SP cells demonstrated significantly greater tumorigenicity than NSP cells in xenograft model of nude mice. Complex decoy oligonucleotide designed to simultaneously target Sox2, Oct4 and c-Myc efficiently suppressed all CSC properties and phenotypes, and minimized the tumorigenic capability of the SP cells and the resistance to chemotherapy. Authors suggested that Sox2/Oct4/c-Myc is a potential anti-pancreatic cancer agent worthy of further studies.
Lin et al. 2014 [88]	OCT- 4 expression is significantly elevated in tumor tissue compared to adjacent noncancerous tissues ($P = 0.005$) The knockdown of OCT4 inhibited the proliferation and invasion of pancreatic cancer cells (Panc- 1) expressing high levels of OCT4, accompanied with decreased expression of AKT, PCNA and MMP- 2
Herreros-Villanueva et al. 2014 [89]	Key embryonic stem cell factors, such as OCT4, NANOG and SOX2, are aberrantly expressed in pancreatic ductal adenocarcinoma Multiple groups have shown this and are compiled in this article.
Assadollahi et al. 2015 [90]	Expression of Oct4, Nanog and Sox2 was studied in cell lines MIA Paca- 2, PA-TU- 8902 and AsPC- 1 and pancreatic cancer tissue Oct4, Nanog and Sox2 expressions were more in the cancer cell lines than normal samples
Shahri & Sayyedal-hosseini 2020 [91]	MIA Paca- 2, PA-TU- 8902 and AsPC- 1 cell lines and pancreatic tumor and non-tumor specimens were studied for Oct- 4 by real-time PCR. Oct- 4 expression was higher in cancer cells compared to normal tissue
Roy et al. 2024 [92]	Mouse pancreatic cancer organoids were studied for Oct- 4, Sox2 and Nanog in response to various therapies [4–8 Gy of radiation, 10 µM of 5-fluorouracil or with 100 µM 3-Bromopyruvate]. Expression of these markers was affected by various treatments suggesting their role in pancreatic cancer growth and both chemo- and radio- resistance

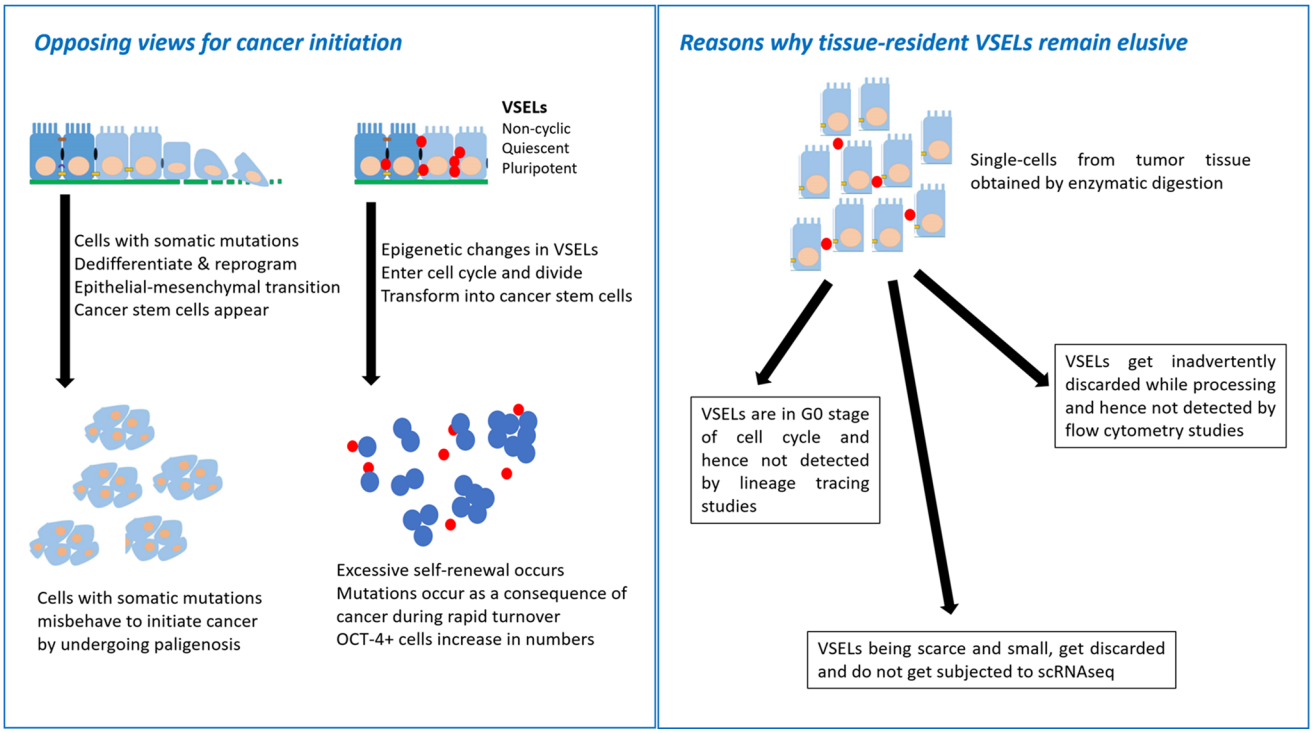

Fig. 5 VSELs initiate cancer but remain poorly acknowledged because of their small size, rare occurrence, and quiescent nature

in numbers of CSCs (epigenetically altered VSELs) and the somatic mutations occur consequently when the CSCs undergo rapid clonal expansion [3].

Conclusions

To conclude, VSEL biology in adult tissues needs to be appreciated rather than proposing potentially misleading concepts by using sophisticated technical tools with obvious limitations. The presence of VSELs amongst epithelial cells in solid tissues is set to usher in a paradigm shift in our understanding of cancer biology (Fig. 5). Rather than initiating paligenosis in differentiated and 'senescent' epithelial cells with a limited life span, exposure to extrinsic/intrinsic insults induces epigenetic changes in VSELs that transition into CSCs, and their excessive self-renewal initiates cancer as discussed earlier [3]. The use of organoids for screening cancer drugs needs to be debated. We need to focus on CSCs that get mobilized into peripheral blood when a tumor starts forming for early detection of cancer [38] and also to get insights into metastases and recurrence [3]. Tissue-resident VSELs are the root cause of cancer initiation, metastasis, and recurrence. Early detection of cancer by studying stem cells in circulation offers better options for reversal and cure when cancer is still a weak disease.

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