Cells, Stem Cells, and Cancer Stem Cells

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Abstract

The stem cell field owes a great deal to the previous work conducted by embryologists and researchers devoted to reproductive medicine. The time is coming when this emerging field will pay off in the reproductive sciences by offering new avenues of understanding gametogenesis and early embryonic development. Human embryonic stem cells are pluripotent cells that proliferate in vitro while maintaining an undifferentiated state, and they are capable of differentiating into most cell types under appropriate conditions. Embryo-friendly approaches have been developed as new methods of obtaining human embryonic stem cells without destroying the embryo. Somatic stem cells have been identified and isolated from numerous adult organs and tissues to create a multipotent and autologous source of cells with established medical indications. Cell reprogramming is now a scientific fact, and induced pluripotent cells, a new pluripotent cell type, have been generated by the overexpression of specific genes from a myriad of differentiated adult cell types. Cancer is now considered a stem cell disease. Cancer stem cells share numerous features with normal stem cells including hallmarks properties such as self-renewal and undifferentiation. Therefore, the actual focus of ovarian cancer research on the cancer stem cell model should generate efficient and personalized treatment designs to improve treatment efficiency.

Keywords
- stem cells
- cancer stem cell
- microenvironment

Stem Cells

Most of the cells in the human body are differentiated and possess a particular function. Stem cells (SCs) are unique cells with the exceptional ability to renew themselves indefinitely by remaining in an undifferentiated state until receiving signals that lead to a differentiated cell type in maintaining tissue homeostasis. These two properties have to be well regulated and are critical in the ontogeny and the proper maintenance of tissues and organs.

SCs are fundamental players in cell biology by allowing tissues to be replenished from freshly created cells throughout their lifetime. The gold standard of a stem cell is the fertilized egg, which is totipotent and generates a complete set of specialized somatic diploid cell types, together with the haploid germline that will be responsible for genetic transmission to the next generation. As the embryo develops, an outer protective membrane of trophectoderm encases a mass of pluripotent stem cells to constitute the inner cell mass (ICM), thus forming one of the first local SC microenvironments during development. Embryonic stem cells (ESCs) are artificially created after the ICM is separated from its niche, and they are cultured in specific conditions by creating a pluripotent SC type that has the ability to originate all the embryonic tissues, except trophectoderm. Somatic stem cells (SSCs) are multipotent cells present in adult tissues or organs that differentiate into a specific cellular lineage. They remain dormant in the G_0 phase and proliferate through asymmetric cell division, giving rise to one daughter SC and to one transit-amplifying cell. Their activation occurs during particular periods of time or after external injury, and their regulation is strictly controlled in their niches.

Niches are protective local microenvironments composed of SCs and neighboring differentiated cell types that secrete

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and organize the extracellular matrix to allow SCs to maintain their unique property of undifferentiation and self-renewal through asymmetric division.

SCs have enormous potential in the biomedical research field, and they are used not only as an in vitro research tool in cellular biology but also as a cellular source for tissue regeneration and cellular replacement therapies. SC research has contributed greatly to knowledge about tissue/organ development from a single cell, tissue engineering, and cellular repair mechanisms. In addition, these features make them an ideal instrument for drug screening and models to study developmental biology. Thus SCs are the core of promising areas such as tissue engineering, gene therapy models, and, finally, cell-based therapies.

Depending on their origin, SCs can be obtained from embryos, fetuses, or adult organisms. However, Japanese researchers[1] have demonstrated that well-differentiated cells can be reprogrammed to the pluripotent SC status and that they can generate a new SC type named induced pluripotent stem cells (iPSs).

ESCs are undifferentiated nonspecialized cells that are established from preimplantation embryos at the cleavage or blastocyst stage. Thus the ESCs deriving from the inner cell mass of a blastocyst present a unique feature such as the ability to replicate indefinitely without cellular differentiation (self-renewal) while maintaining an infinite proliferation rate in culture and the capability to differentiate in vitro into three germ layers: ectoderm, endoderm, and mesoderm. Furthermore, ESCs injected into host embryos are capable of contributing to the germline in the chimeric animals generated. Moreover, after testicular injection in nonobese diabetic/severe combined immunodeficient mice, they produce teratomas. In addition, the generated ESC lines maintain a normal karyotype, genomic stability, and express high levels of telomerase activity. These properties, defined as "stemness," outline ESCs as a potential source of specialized cells for future cell replacement therapies.

Since Thomson's group isolated ESCs from the ICM of early human embryos and obtained the first successful human embryonic stem cell (hESC) line, derivation of hESC cell lines has evolved from the isolation of the ICM through diverse methods such as immunosurgery[2] and laser dissection[3] by micromanipulation techniques through a laser drilled in the zona pellucida or through a whole embryo culture.[4] Irrespective of the method used, embryo destruction was mandatory and the cells obtained were transferred to fibroblast feeder layers, which serve as a support and supply of growth factors. However, successful derivation methods without fibroblast feeder layers[5] under conditions known as "feeder free" and "serum free" have been reported, and they help eliminate the risk of xeno-contamination during the in vitro derivation process. Furthermore, translation of the in vitro fertilization clinic procedures has clearly improved the derivation of hESC lines to avoid embryo destruction by following a single-cell biopsy method at the cleavage stage that does not interfere with embryo viability.[6,7] Actually, the derivation of clinical-grade hESC lines can be achieved without embryo destruction in a cellular culture system, which uses a chemically defined medium free of animal products (►**Fig. 1**).

SSCs, also known as adult stem cells, are able to replicate asymmetrically by generating progenitor cells with a finite division capacity that finally differentiate into mature cell types. Thus in each tissue, adult SCs provide a source of differentiated cells to preserve a homeostatic cell turnover status due to both tissue demand and/or injury consequence.[8,9]

SSCs have been successfully isolated from different adult tissues (e.g., bone marrow,[9] adipose tissue,[10] umbilical cord blood,[11] connective tissues of the dermis[12] etc.) through various techniques based on phenotypic markers including cell surface markers and nonspecific techniques such as the high-level activity of adenosine triphosphate (ATP) binding cassette (ABC) transporters.[13] These adult SCs present cellular plasticity, which is clinically useful in SC-based therapies to generate differentiated cell types. Given their plasticity and accessibility, many studies are exploring the clinical potential of adult SCs that are capable of differentiating in a wide range of different lineages in vitro and in vivo obtained from the same or a different germ layer[14–18] (►**Fig. 2**).

However, the SSCs present in each tissue are few in number and have a limited long-term proliferation capacity in culture without undergoing differentiation.[19,20] This is a major limiting factor in using adult SCs for both research and clinical applications.

Interest in SCs is an undeniable fact given their innate therapeutic potential in regenerative medicine. However, practical applications have gradually come about, partly due to technical problems and to the ethical and moral debate about their use. In an attempt to obtain an alternative source of pluripotent cells without ethical and religious conflict, in 2006, Takahashi and Yamanaka[1] identified the factors responsible for reprogramming somatic cells toward a pluripotent phenotype. The publication of this novel protocol assumed that the factors responsible for maintaining the pluripotency status in ESCs were just as well capable of inducing this capability in somatic cells.

Initially, 24 factors were selected as candidates based on their functions and their specific expression profile in mouse ESCs. For the purpose of finding the best combination, they were introduced through a retroviral vector into mouse embryonic fibroblasts (MEFs). Finally after various combinations, the authors just cited demonstrated that only four of these factors were required to induce iPS from MEF colonies: Oct3/4, Sox2, c-Myc, and Klf4. The iPS cells generated presented morphology, growth features, and functional properties indicative of pluripotency, and they also expressed a significant number of pluripotency markers similar to ESCs. Nonetheless, the first iPS cells presented a lower expression level of transcription factors, as well as differences in the epigenetic profile of promoter regions, compared with ESCs.

Despite the reprogramming process requiring subsequent modifications of the induction protocols to obtain fully well-reprogrammed iPS, this finding proved to be the milestone in the pluripotency rule, and it demonstrated that cellular reprogramming is feasible[1] and applicable in human

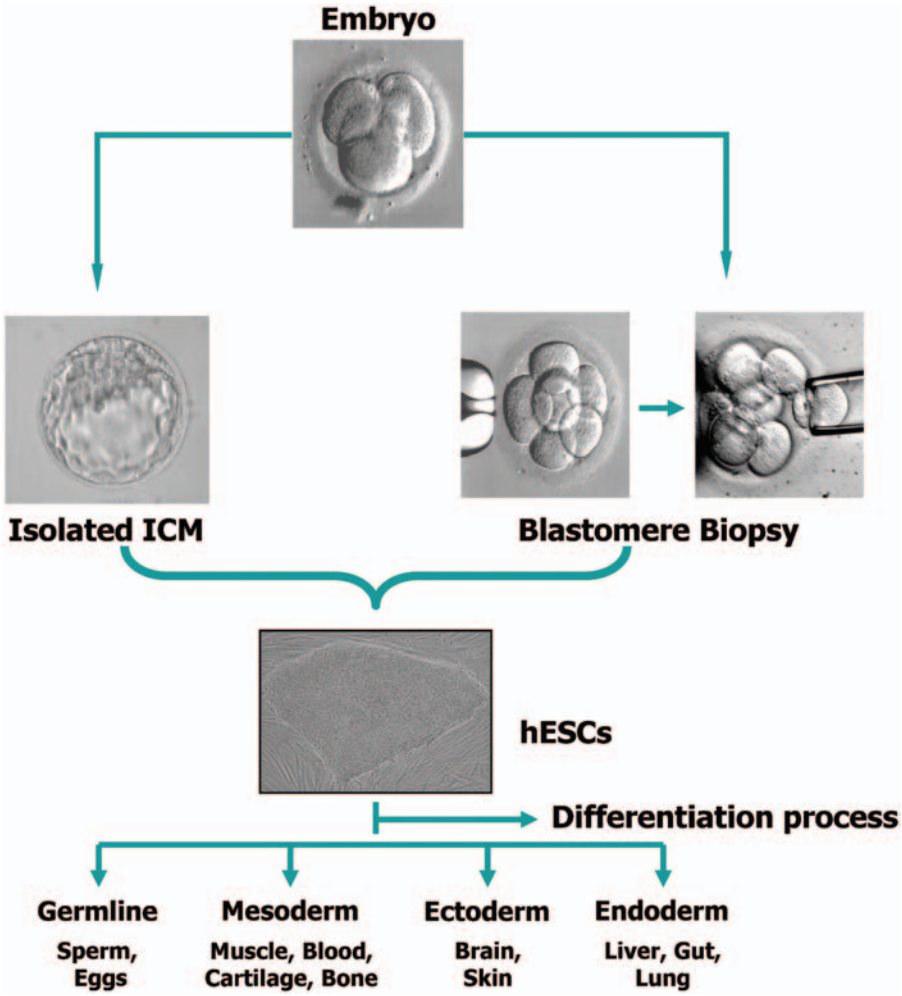

Figure 1 Schematic diagram of the derivation and differentiation of human embryonic stem cell (hESC) lines. Pluripotent cells are isolated from either the inner cell mass of preimplantation blastocysts or single blastomeres at the four- to eight-cell embryo stage. Isolated cells are plated in defined hESC medium with or without feeder cell layers to proliferate and select for pluripotent cells. The generated hESC lines are able to differentiate into all the tissues from all three embryonic germ layers and the germline. ICM, inner cell mass.

cells.[21–23] Currently, however, iPS cells generation focuses on development safety and efficient methods before reaching the real clinical approach. Thus therapeutic iPS cells should be generated through nonintegrative methods to guarantee the absence of exogenous sequences inserted into the genome with a view to excluding the possibility of mutagenesis.

In fact, iPS cells can be generated from differentiated somatic cells with a few defined factors.[24] Recent studies have shown that *p53* inactivation (primary tumor suppressor), which regulates the cell cycle, avoids genome mutation and conserves its stability, thus preventing cellular aberrant division via apoptosis or senescence. Experimental silencing *p53* through deletion or knockdown improves the efficiency reprogramming rate and reduces the number of factors required to achieve it. Hence, silencing *p53* not only optimizes both the number of reprogrammed cells and the time required for the process. Yet despite *p53* inactivation possibly being key to increased efficiency,[25–27] this strategy may increase the likelihood of either generating cells with an unstable genome or inducing malignant transformation.

A large number of somatic cells has been reprogrammed by applying different approaches[28–31] including direct transdifferentiation from one lineage to another[32] and disease-/patient-specific reprogrammed cells,[33–35] which represent an invaluable possibility of generating cell types of interest to be applied to autologous cell replacement therapies (e.g., the development of specific disease models) (►**Fig. 3**).

iPS cells are definitely a remarkable achievement, although their clinical application is presently limited due to serious obstacles in biosafety terms. Therefore, their clinical uses should wait until accompanied by appropriate differentiation protocols, antitumoral safety, and a proper functionality posttransplantation test.

Cancer Stem Cells

The traditional way of explaining cancer initiation and progression is through the accumulation of somatic mutations.[36] This dominant concept implies that cells might progressively induce the loss of specific tissue features with each mutation

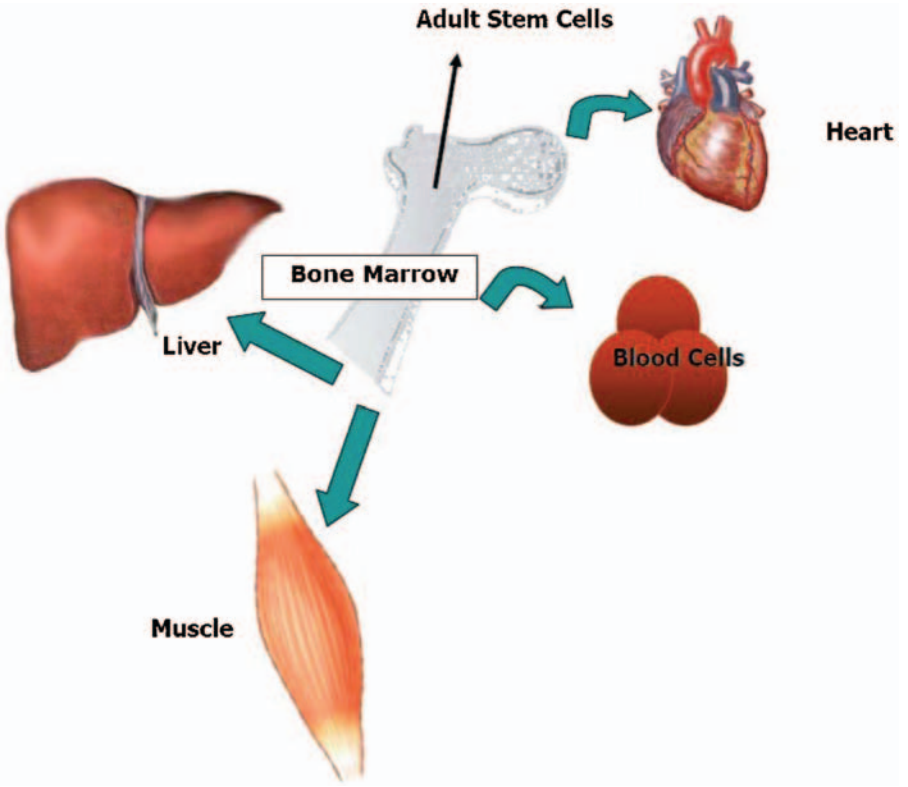

Figure 2 Adult stem cells have been identified in many organs and tissues, responsible for maintaining and repairing the original tissue in which they are found. They form specialized cell types through differentiation pathways to establish a stable cellular turnover. They are also able to differentiate into cell types from different germ layers through a process known as plasticity.

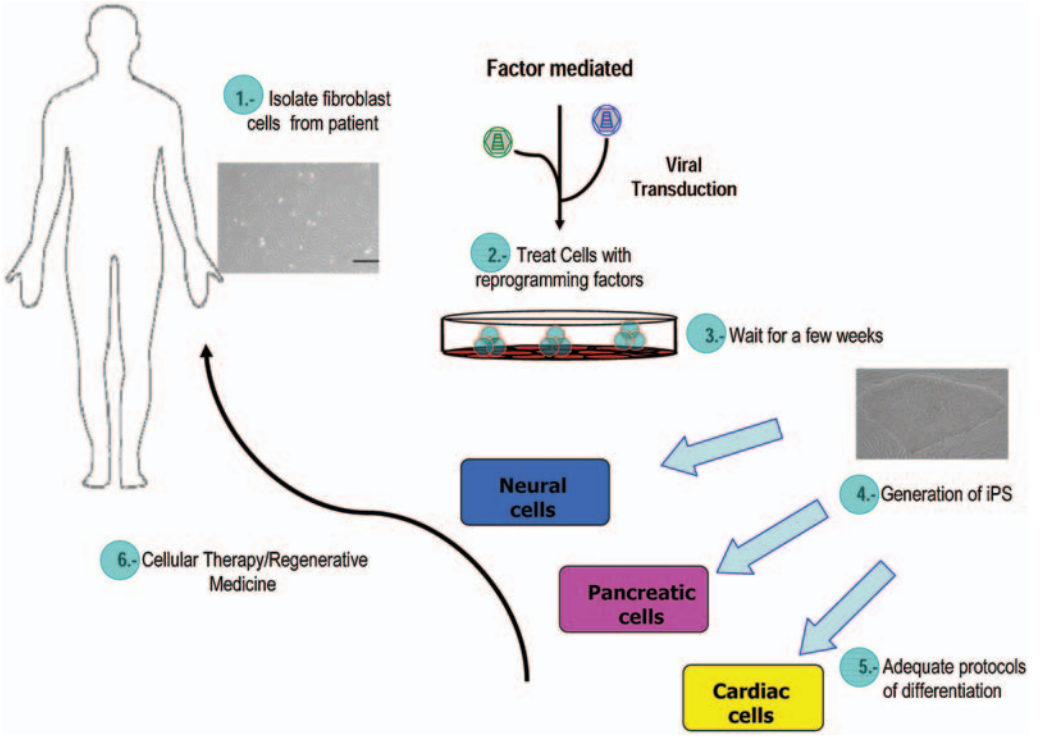

Figure 3 Generation-induced pluripotent stem cell (iPS). Diagram shows the protocol to obtain iPS cells from a patient to generate iPS lines. These pluripotent cells might be used as both autologous cell replacement therapies and disease-specific iPS lines that mimic the donor's disease. Their useful applicability in drug screening toxicity testing and in developing and improving therapies through reproducing human disease in culture helps evaluate progression and response.

entering a dedifferentiation state and regressing to a primitive phenotype. This transformation might guide uncontrolled proliferation and hence increase the number of affected cells. Once transformed into cancer cells, not only does their proliferative capacity increase, but also their tumor formation ability. Theoretically, therefore, in this stochastic model, once a random mutation and a subsequent clonal selection have taken place, each cell would be equally capable of forming a new tumor. However, findings relating to the cellular hierarchy and tumor heterogeneity responsible for the different phenotypes advocating original tissue features suggest that this model may be excessively simplistic.

Several critics have argued against the somatic stochastic theory and have instead favored an alternative hypothesis. Cancer stem cells (CSCs) is a model that proposes a hierarchically tumoral structure that is similar to normal tissue and characterized by self-renewal subpopulation cells termed tumor-initiating cells (TICs) that possess a stemness profile responsible for the generation of a large population of proliferative cells that are ultimately responsible for tumor development.[37]

Remarkable considerations have reinforced the CSC hypothesis because the tumor can be initiated from a single cell capable of recapitulating tumoral heterogeneity, constituted by different cell types including a subset of the TIC population responsible for maintaining tumoral growth and the rest of all the heterogeneous lineages of cancer cells constituting the tumoral bulk with limited self-renewal capacity. Thus the complete phenotype of the primary tumor is created, which contrasts with the stochastic cancer development model and proposes that all cancer cells have the equal potential to generate a tumor (►**Fig. 4**).

An association between normal SCs and CSCs is coherent because they share many features and molecular mechanisms regulating the SC function including self-renewal, undifferentiation, long-term survival, organization into a specific hierarchy, and differentiation capacity.

Under normal conditions, the regulation process, with the niche established through paracrine signaling pathways, controls SC and CSC's self-renewal capacity. Hence, the dynamic interactions of stromal cells within a microenvironment may affect tumor development.[38] These interactions between CSCs and the niche involve the activation of inflammatory responses and, simultaneously, epigenetic modifications such as DNA methylation and histone modification patterns, and genetic transformation, which are essential in CSCs' biology because they are ultimately responsible for tumor heterogeneity.

The CSCs have been isolated from leukemia[39] and different solid tumors, such as breast cancer[40] and even ovarian cancer.[41,42] In addition to the stemness profile previously mentioned, they present other common characteristics: (1) a distinctive profile of surface markers,[37] (2) increased aldehyde dehydrogenase activity,[43] and (3) chemoresistance to anticancer agents due to efflux pathways.[44,45] Thus these properties imply an important clinical implication of CSCs in cancer recurrence.

Normal cellular turnover depends on the adequate arrangement of the events regulating the activation of SCs, which is driven by different signaling pathways including *Hedgehog* (Hh), *WNT, NOTCH,* and *BMP*,[46] which regulate the balance between SC renewal and cellular differentiation within the microenvironment, modulated by epigenetic and genetic events.

Cancer Stem Cells Chemoresistance

Standard chemotherapy induces DNA damage as an approach to induce cellular death. However, SCs are generally quiescent with a great DNA repair capacity, and they have developed survival mechanisms through their resistance to apoptosis due to the expression of Bcl-2 family members and to inhibitors of apoptosis.[47] For these reasons, they possess resistance mechanisms against conventional cytotoxic chemotherapy. Therefore, this mechanism that enables the protection of healthy SCs should, in CSCs, make them less susceptible to conventional therapies. One of the most well-known CSC resistance strategies involves cell cycle kinetics remaining in a quiescent state, which makes them less susceptible to the cytotoxic effects of compounds designed against these cells, with a faster division rate and shorter cell cycles.[48]

The overexpression of membrane-bound multidrug efflux resistance transporters is another chemoresistance mechanism. Ovarian cancer patients who have developed resistance to the platinum compound are a well-characterized model. Efflux transporters, such as the *ABCB1* (*MDR1* or P-glycoprotein) and *ABCG2/BCRP* (breast cancer resistance protein, or *BCRP*) members of the ABC family, constitute a cell surface drug-resistance marker (ATP-binding cassette) responsible for a lower platinum concentration in the cell that proves useful in isolating and characterizing ovarian CSCs.[49] Furthermore, it is considered a prognosis marker for disease progression in advanced ovarian cancer.[50]

Ovarian Cancer Stem Cell Biology

Ovarian cancer is the most lethal gynecologic malignancy. As a result of unsuccessful screening methods, more than half of ovarian cancer patients are diagnosed in advanced stage III or IV. Standard ovarian cancer treatment is based on cytoreductive surgery followed by platinum/taxane cycles. Unfortunately, these patients present a recurrence rate of 70% after the initial treatment, and the overall 5-year survival rate of patients diagnosed with distant disease is only 30.6%.[51]

Most reports indicate that ovarian cancer arises from the ovarian surface epithelium, although there is reported evidence that blames the fallopian tube.[52] Ovarian cancer is composed of a heterogeneous group of tumors that are classified into serous, mucinous, endometrioid, and clear cell. The epithelial-mesenchymal transition (EMT) is involved in the malignant transformation of this tumor. The EMT regulatory program confers the ability to detach from the primary bulk through the loss of cell adhesion properties to provide stemness properties including the invasive features

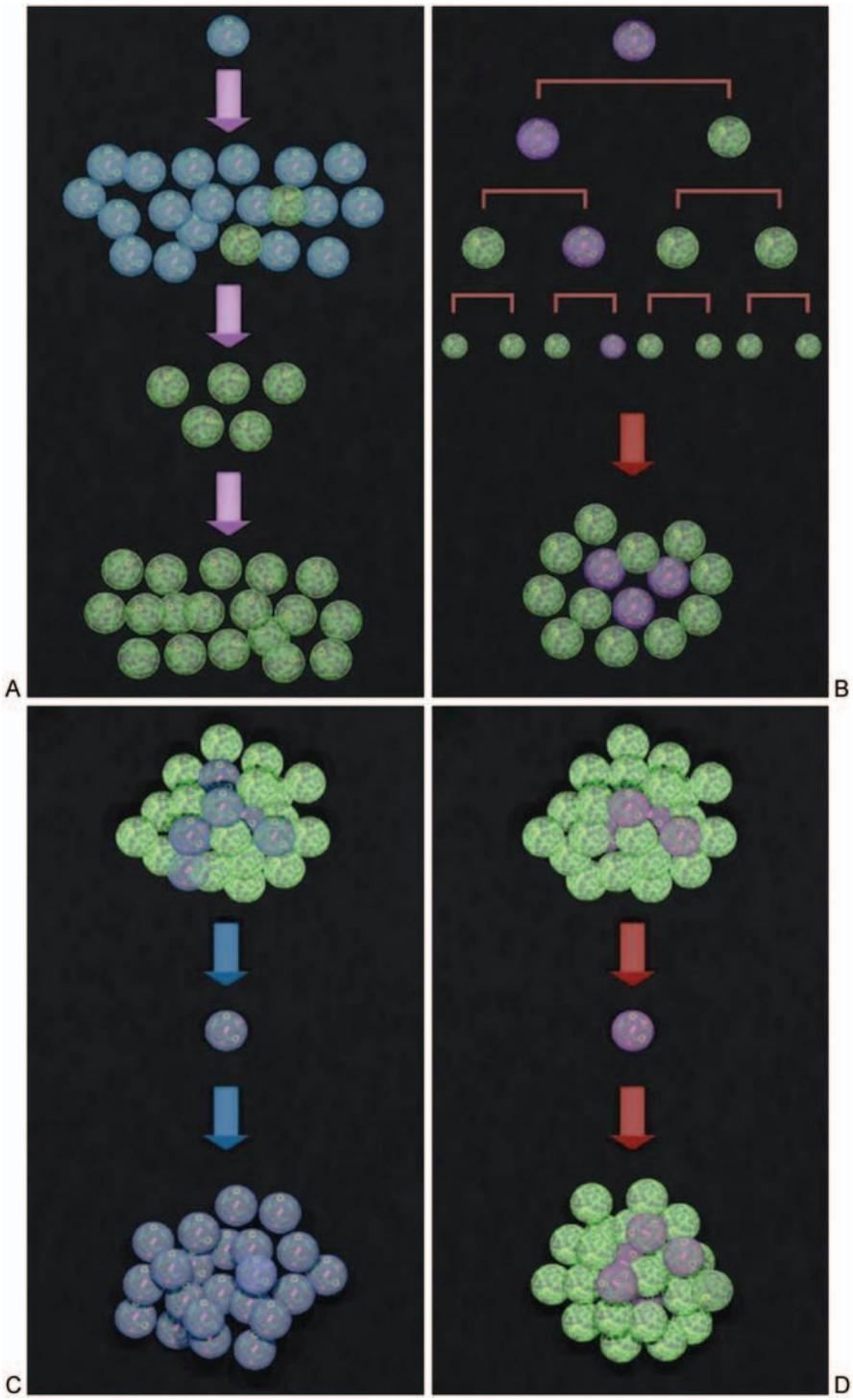

Figure 4 Stochastic model versus cancer stem cells model in solid cancer. (A) In the stochastic selection hypothesis, cancer might begin from any mutated somatic cell. (B) The cancer stem cells (CSCs) model implies hierarchical cellular organization inside the tumor. (C) A stochastic model shows that a cancer cell has the potential to proliferate extensively and not only replicates phenotype complexity. (D) In the CSCs model, one single cell completely recapitulates the heterogeneous parental tumor phenotype.

of cancer cells. In other words, the conversion of epithelial cells into mesenchymal cells through morphological modifications and the acquisition of a migratory phenotype result in increased invasion and metastasis through transcription factors such as *Snail* and *Slug*. The upregulation of these transcription factors triggered in response to radiotherapy and chemotherapy induces the transcriptional repression of the proapoptotic *PUMA/BBC3*, *ATM*, and *PTEN* genes involved in *p53*-mediated apoptosis, leading to improved cell survival. Simultaneously, *Snail* and *Slug* not only lead to the transcriptional activation of self-renewal genes, including *NANOG*, *HDAC1*, *TCF4*, *KLF4*, *HDAC3*, *GPC3*, but also involve the

activation of other SC master regulators such as *OCT4, BMI1*, and *NESTIN*.[53] Therefore, *Snail* and *Slug* are responsible for increased resistance to chemotherapy drug treatment, and they stimulate cell metastases and recurrence of ovarian cancer.

p53's normal function is associated with favorable results in chemotherapy and improved clinical outcomes in ovarian cancer patients.[54] *p53* regulates apoptosis through different genes including *NOXA*, *BAX,* and *PUMA/BBC3*. Resistance to cisplatin is a major cause of treatment failure in human ovarian cancer; *p53* is required for cisplatin treatment to induce apoptosis in ovarian cancer cells and depends on the induction of *PUMA/BBC3*.[55] *The PI3K/AKT* cell-signaling pathway, crucial for normal cell growth, is commonly overexpressed in ovarian cancers. It is associated with tumor aggressiveness, genome instability, and cellular invasion and migration, and therefore, compromises the efficiency activity of both *PUMA/BBC3* and *p53*, thus providing an additional chemoresistant phenotype to cell proliferation and survival in ovarian cancer.

The microenvironment is a crucial factor implicated in malignant cell development. Cancer cells typically capture more glucose to produce ATP through aerobic glycolisis.[56] This effect is associated with the triggering of oncogenes (e.g., *RAS, MYC*) and mutant tumor suppressors (e.g., *p53*). Besides oncogenes, hypoxic conditions might independently regulate glycolysis through hypoxia inducible factor-1α and factor-2α (*HIF-1α, HIF-2α*), probably as a result of adaptation to low-oxygen environments within tumors. Thus it is important to highlight the microenvironment role because the hypoxia level within a tumor correlates with critical signaling pathways such as *NOTCH* and *BMP*,[57] which have demonstrated that hypoxia not only alters cellular energy metabolism and angiogenesis but also influences the proliferation and maintenance of undifferentiation and resistance to chemotherapy.

Various wide genomic analyses of epithelial ovarian cancer stage II through IV have acknowledged that high-grade serous ovarian adenocarcinomas are characterized by *p53* mutations in 96% of cases, together with commonly mutated genes such as *NF1*, *BRCA1*, *BRCA2*, *RB1,* and cyclin-dependent kinase 12 (*CDK12*).[58] Signaling analyses have indicated that *NOTCH* and *FOXM1* are significantly involved in serous ovarian cancer pathophysiology.[58]

Studies using comparative genomic hybridization have demonstrated that *PI3K* and its downstream effectors *AKT1* and *AKT2* are significantly amplified in aggressive ovarian carcinomas.[59] Tumor suppressor gene *PTEN,* which antagonizes the *PI3K-Akt/PKB* pathway, has also been seen to be a negative regulator by dephosphorylating *PIP3* and the subsequent downregulation of the *PI3K-Akt/PKB* signaling pathway. Moreover, *PTEN* mutations have been found only in endometrioid ovarian tumors. The absence of *PTEN* mutations in other histologic subtypes supports the notion that ovarian cancers arise through distinct developmental pathways.[60]

Accumulated evidence demonstrates that DNA methylation patterns of cancer cells are significantly altered if compared with normal cells. CpG islands hypermethylation in DNA has been associated with not only poor ovarian cancer prognoses but also with the silencing of major tumor suppressors such as *BRCA1/2*[61], *DLEC1*,[62] *OPCML*, *TES*, and *RASSF1A*.[63] Thus these epigenetic changes, which do not involve changes in the DNA sequence, are implicated in malignant transformation and progression.

DNA methylation events, which involve the addition of a methyl group in the cytosine inside CpG sequences, have been associated with histologic and clinical features of ovarian carcinomas. *SFN,* an inhibitor of G2/M progression of cell cycle progression, is frequently methylated in ovarian clear cell carcinomas. *WT1* is a tumor suppressor that plays an important role in cellular development and cell survival in clear cell ovarian tumors.[63]

Development of ovarian cancer drug resistance might also result from DNA methylation, which induces the transcriptional silencing of drug response genes, or even the opposite situation in which DNA hypomethylation could induce the activation of oncogenes[64] and multidrug transporters (i.e., *ABCG2/BCRP*).[65]

Histone modifications are another epigenetic regulator mechanism. Acetylation in histones H3 and H4 is associated with transcriptionally active sequences; hypoacetylation leads to chromatin condensation that correlates with transcriptional silencing. In line with this, the hypoacetylation of histones H3 and H4 suppresses the *DLEC1* expression in ovarian cancer and H3 acetylation reduces DNA methylation, which triggers the expression of claudin-4 (an essential protein in tight junction formation) that is frequently upregulated in ovarian tumors.[66]

The epigenetic status influences not only cancer development but also the stemness prolife, differentiation and the quiescent state, whereas the microenvironment is also crucial in this process. Varied conditions may have an impact on the niche and its physiology, and include stress, aging, exposure to cytotoxic substances, and so on. However, selective pressures of these genetic and epigenetic aberrations are required to drive and finally establish clonal expansion and cancer.

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