Availability of Pluripotent Stem Cells from Normal Cells in Cancer Science

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Abbreviations

ADM	Acinar to ductal metaplasia
AFP	Alpha-fetoprotein
AML	Acute myeloid leukemia
CAFs	Cancer-associated fibroblasts (CAFs)
CEA	Carcinoembryonic antigen
CFU	Colony-forming unit
ciPSCs	Converted iPSCs
CK19	Cytokeratin-19
CM	Conditioned medium
Colla1	Collagen type I alpha 1 (Col1α1)
CSCs	Cancer stem cells
Dox	Doxycycline
ESCs	Embryonic stem cells
FSP1	Fibroblast-specific protein 1
GPC3	Glypican-3
HSCs	Hematopoietic stem cells
iCSCs	Induced cancer stem cells
iPSCs	Induced pluripotent stem cells
LIF	Leukemia inhibitory factor
miPSCs	Mouse iPSCs
NACs	Non-adherent cells
NPCs	Neural progenitor cells
PDGFα	Platelet-derived growth factor-α
PTEN	Phosphatase and tensin homolog
α-SMA	α-smooth muscle actin
PRCC	Papillary renal cell carcinoma
PDAC	Pancreatic ductal adenocarcinoma
RNA-seq	RNA sequencing
SDF-1α	Stromal-derived factor-1α
TGFβ1	Transforming growth factor β1
TOFT	Tissue organization field theory
VPA	Valproic acid

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15.1 Introduction and a Short History of Cancer Research

Attempts to understand the origin and cause of cancer are back into the earliest period of life when human began to observe diseases. Throughout history, a gradual understanding of tumors by the researchers and the different treatments, such as simple herbal, salt mixtures, and primitive surgery techniques, was applied. The first available documented description of cancer as a disease is back to 3000 BC, founded in the ancient medical text Edwin Smith Papyrus, which contained a description of breast cancer as a deadly disease. The ancient Egyptian also tried to treat this disease with arsenic paste. A notable progress, after that, was introduced by Greeks around 400 BC, where Hippocrates began to give more details about cancer; he gave the name of "cancer" to the disease as he believed the similarities between the disease and moving crab and described the disease as a natu-

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ral cause. More comparison between crab and cancer, especially the crab's ability to adhere by its claws to a different direction, was done. At the same time, there were attempts to treat cancer by using a mixture of egg and honey (Hajdu 2011).

Much data have also been documented by different cultures, including ancient Chinese, Indian, Persian, and Muslims in this regard. When Baghdad City was the center of the scientific world, remarkable information about cancer and its treatments were documented by different scholars such as Avenzoar and Avicenna, who described cancer in his famous book *The Canon of Medicine*. Some of his special notes were that some cancers are more common in women and internal tumors could be removed by specific surgery techniques such as polypectomy. He also suggested that external tumors are possible to treat than internal ones that grew continuously and became difficult to treat (Golzari et al. 2013). During the period between 1500 and 1700, the progress in the cancer field was due to surgery and pathology specialties describing the difference between benign and malignant tumors and distinguishing sarcoma from carcinoma. At that time, lung tumors were also diagnosed either as primary or secondary tumors coming from other parts, and their treatment was more difficult. The tumor's origin was also introduced by Deshaies Gendron, who proposed that cancer was arising from the transformation and continuous growth of different solid structures of the body. At the same time, chronic inflammation and tobacco were also suggested as causes of cancer. More types of cancer have been documented in this period through notes of Theophilus Bonetti, who recorded 43 case reports on colon, pancreatic, liver, lung, stomach tumors, and so on (Manchester 1997).

The first attempt to transplant human cancer sample into animals was performed by Bernard Peyrilhe. He injected human breast cancer extracts into a dog in 1775 as the first experiment in this direction even before developing the concept of cells (Androutsos and Karamanou 2009). From the eighteenth century up to the beginning of the nineteenth, cancer was also presumed to rise from chronic exposure to environmental agents such as soot, tobacco, coal tar, and hot paraffin or flow obstruction of body fluids. In this period, cancer terms, such as "soft cancer" referring to lymph glands and soft tissue cancers and "metastasis" referring to invasion and spreading of tumor far from original places, were introduced (Anttila and Boffetta 2014).

One of the most distinguished findings that impacted cancer research was the cell theory established by Theodor Schwann, who proposed cells as blocks of human and animal tissues. After that, Muller described cancer as groups of abnormal cells and stroma. Cell theory introduced the routine use of microscopes for medical research giving more details and descriptions about tumor tissues and classifying cancers depending on microscale features illustrating cancer cells (Ribatti 2018). In the second half of the nineteenth century, much of the groundwork done by the researchers enormously contributed to the progress and advancements in the cancer field. Coley injected toxins to support the patient immune system against tumors. Novinsky successfully transplanted dog and rat tumors into healthy animals. Controlled exposure to X-rays was being used to treat cancers. Ehrlich introduced the term "chemotherapy," that worked to develop chemical compounds as drugs for cancers and suggested that the tumor consisted of chemically resistant and sensitive cells (Hajdu 2012).

At the beginning of the twentieth century, a remarkable experiment by Peyton Rous showed the ability of cell-free filtrates from hen sarcoma to induce cancer in another hen, and the cancer-causing agent was later identified as a virus named the Rous sarcoma virus. At the same period, Theodor Boveri proposed the basics of somatic mutation theory. In this theory, Boveri assumed that cancer occurred due to mutations, "abnormal chromosomal rearrangement," which lead to cell proliferation and cancer initiation (Di Lonardo et al. 2015). During the twentieth century, cancer research grew significantly wherein many groundbreaking discoveries were made. Yamagiwa was the first to succeed in inducing an invasive skin cancer in animals by applying crude coal tar, which was considered as a mixture of pro-inflammatory chemicals (Yamagiwa and Ichikawa 1918). X-rays exposure was found to induce skin cancer in radiation workers (Shore 1990).

These early findings allowed observing differences between malignant and normal cells and establishing cancer cell lines. Since Yamagiwa's results promoted the investigation of carcinogenesis, the effects of hundreds of chemicals were assessed during the decade after the discovery in various research laboratories. Simultaneously, Warburg found that cancer cells consumed glucose at a rate higher than normal cells, a phenomenon called "the Warburg effect," which formed the basis of cancer metabolism. Watson and Crick's discovery of DNA structure had enormous influence on molecular oncology (Liberti and Locasale 2016). In 1953, the experiments by Helene Toolan were among the first successful attempt of human cancer sample transplantation into animals. In these experiments, Toolan reported that human tumors could successfully grow and proliferate in cortisone-treated laboratory animals (Xu et al. 2020). Breast cancers were linked to the familial breast carcinoma-related gene mutations such as BRCA1 and BRCA2, besides patients' family history. Viruses were suggested to be responsible for transforming normal cells to cancer cells by viral-derived genes, so-called oncogenes.

On the contrary, tumor suppressor genes, such as retinoblastoma Rb1 and P53, inhibited cell proliferation and affect cell cycle. The loss of the p53 gene and its mutations were linked to the malignant transformation of normal cells. Many oncogenes and suppressor genes have already been identified since then (Buchholz et al. 1999; Miller and Stebbing 2018).

The accumulated data focusing on the mutations and chromosome abnormalities guided the researchers to the somatic mutation theory. In light of this theory, the normal cells required multiple mutations (3 to 7) for cancer initiation, and its subsequent progression. Therefore, tumor formation was proposed to depend on a series of mutations through multiple intermediary stages (Fisher 1958). Cancer growth was shown to rely on blood vessels when Folkman and his colleagues provided an evidence that neovascularization was vital for solid tumor growth. The term "tumor angiogenesis" began to be used widely in cancer research (Ribatti 2008). The discovery of nude mice, which were hairless, immunocompromised mice lacking thymus glands, accelerated cancer research by enabling to simply reestablish tumor models in animals without drug or radiation since nude mice were mutant defecting the immune system (Neff 2016; Szadvari et al. 2016).

On the same note, isolated from a wide range of cancer types, various cancer cell lines have been developed and used for in vitro or in vivo experimental models, which were extensively used to investigate the cell characteristics, such as tumorigenesis, drug resistance, and metastasis in different cancer types. However, these cells often fail to provide insights for tumor development and progression due to the alteration of characters after years of careless maintenance in vitro since their genomic and/or morphological characteristics have changed over time. If the preclinical experiments are carelessly performed under this situation, the following clinical phase testing will encounter frequent failures. This lack of translational success is often ascribed to multiple parameters, including tumor heterogeneity, diverse and complex cellular interactions, and limited availability and access to the in vitro the 3D tumor microenvironment models to mimic in vivo microenvironment (Ben-David et al. 2019).

For decades, somatic mutation theory remained the predominant theory of cancer origin, thus providing a considerable number of studies based on this notion. This bias decreased the opportunity to investigate other hypotheses of the origin of cancer in a more sophisticated way or neglected sometimes in favor of somatic mutation theory (Brucher and Jamall 2016; Soto and Sonnenschein 2014). At the end of the twentieth century, the epigenetic field has begun to emerge, and cancer tissues were subjected to epigenetic analysis. Epigenetic changes refer to the changes in genetic information rather than the DNA sequence, such as mutations (Brucher and Jamall 2014b). Epigenesis includes DNA methylation and/or histone methylation/acetylation, etc. In early 1980, the epigenetic changes were reported to involve oncogenes and tumor suppressor genes to regulate their expression, thus altering the resultant phenotypes. Therefore, epigenesis was assigned a predictive role in cancer theranostic applications (Baylin and Jones 2016). Feinberg et al. found that specific genes in human tumor tissues were hypomethylated compared to those in normal adjacent tissues.

A significant difference in DNA methylation was found between different human malignant tumors, benign tumors, and normal tissues (Feinberg and Vogelstein 1983). Cancer epigenetics is primarily focused on activating oncogenes and inactivating cancer suppressor genes. Epigenetic alterations due to environment or aging have also been linked to carcinogenesis and possible role to initiate cancer. Recently, new hypotheses have been put forward regarding the mechanism underlying cancer initiation. A large number of scientists began to think that cancer is a tissue-based disease instead of being a cell-based disease. Carlos Sonnenschein suggested the tissue organization field theory (TOFT) as one of the cancer initiation mechanisms where abnormal interaction between tissue microenvironment and different types of cells, i.e., stromal or epithelial cells, could result in cellular transformation and cancer initiation. This is not necessarily dependent on mutations or clonal dominance of mutant cells (Soto and Sonnenschein 2011). In this context, biophysical forces and interactions between cells and tissues are pivotal factors in the carcinogenesis process. This insight is also supported by a long history of studies and the accumulation of evidence supporting the crucial roles of chronic inflammation in initiating cancer.

Different stimuli and pathogens could induce chronic inflammation, which is a long-term disruption of homeostasis in tissues. Abnormal secretome profile and cell-to-cell interactions could eventually lead to cellular transformation. Experimental exposure of chemicals to different animal tissues for an extended period was among the first cancer induction experiments. Some viruses such as hepatitis B and C and bacteria such as helicobacter pylori were linked to different types of cancers such as the liver and gastric cancers. The link between these factors is the disruption of chronic inflammation-inducing signaling, which seems to be crucial in the early stages in cancer development (Brucher and Jamall 2014a, 2019). Figure 15.1 summarizes major discoveries and events in cancer research.

Different tools exist for cancer studies, such as cell lines, patient-derived xenografts, and different experimental animal models. However, it is insufficient to encompass all the cancer initiation mechanisms. Therefore, additional disease modeling strategies are needed to complement existing techniques in cancer research. In this regard, induced pluripotent stem cells (iPSCs) could go a long way provide a powerful tool in this area.

15.2 Cancer Stem Cells in Cancer Science

The concept of cancer stem cells (CSCs) is long-standing and dates back to the nineteenth century when Julius Cohnheim mentioned the similarity between cancer cells and embryonal cells. Cohnheim suggested that the origin of

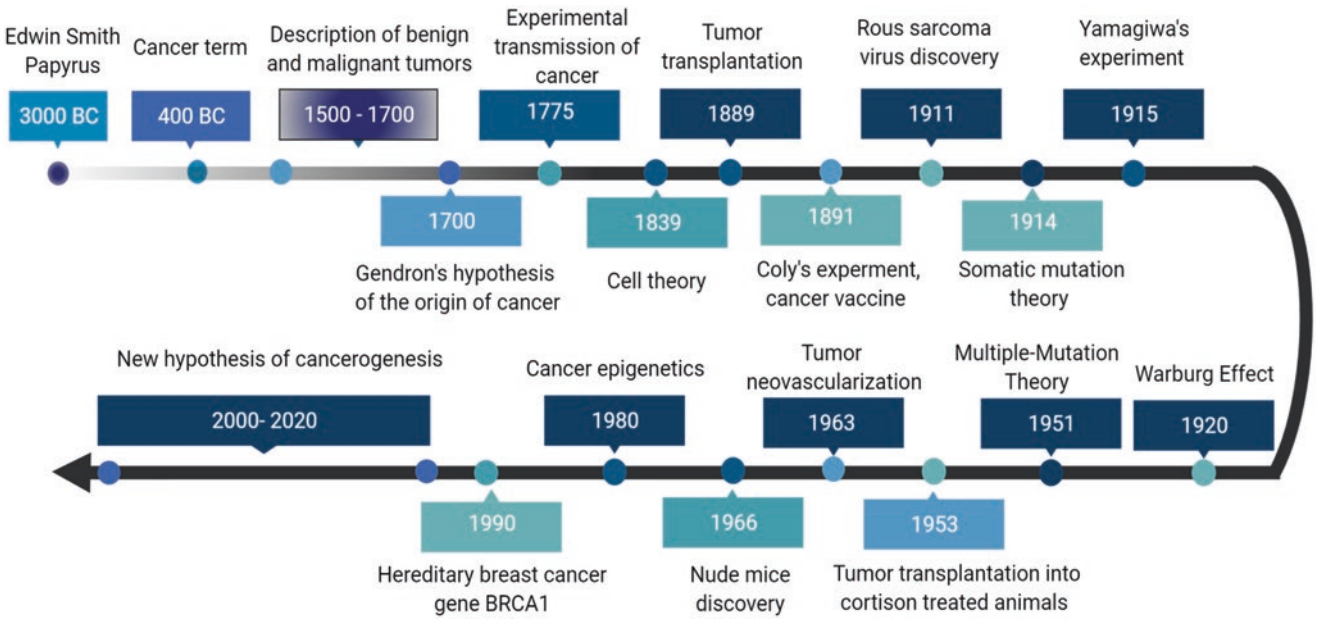

Fig. 15.1 Timeline of some major events and discoveries in cancer research history

cancer is from cells misplaced during embryonal development and/or retaining the embryonal characteristics. In 1977, Hamburger and Salmon developed a cell culture protocol for the tumor stem cells by culturing tumor cells in semisolid conditions where some of tumor cells selectively formed colonies, while cells from healthy volunteers failed to colonize under the same set of culture conditions (Capp 2019). CSCs were isolated for the first time by John E. Dick from acute myeloid leukemia (AML) specimens. In this study, a rare subpopulation, identified using $CD34^+/CD38^-$ expression, showed the ability to initiate cancer upon injection into immunodeficient mice. On the other hand, $CD34^+/CD38^+$ and $CD34^-$ cells, the majority of myeloid leukemia cells, failed to give the same results as $CD34^+/CD38^-$. Therefore, $CD34^+/CD38^-$ cells were proposed as CSCs with their self-renewal and colony-forming ability. In this study, Dick also suggested the hierarchy of leukemia stem cells, which gave more mature cells in AML colonies (colony-forming units, CFU), which had less proliferative potential when cultured for a long time. Dick observed that both normal hematopoietic stem cells and leukemia stem cells were sharing the same phenotype, $CD34^+/CD38^-$. Therefore, he suggested comparing the gene expression between them to find significant genes and markers for leukemia stem cells. Dick focused on hematological malignancy because many hematological malignancies did not have an appropriate in vitro assay besides limited proliferation capacity of the responsible cells, unlike solid tumor cells, which could be easily cultured and maintained in vitro using the available optimized protocols (Bonnet and Dick 1997).

At the end of the twentieth century, the concepts of tumor heterogeneity and CSCs were prevalent, and CSCs were successfully isolated from hematological malignancies. In 2000, the first report of isolation and identification of CSCs in solid tumors came out. In this study, Al-Hajj et al. found that a minority of breast cancer cells, characterized as $CD44^+/CD24^{low}/lineage^-$, could form tumors at a very low number of 100 cells when injected in immunodeficient mice (al-Hajj et al. 2003). The authors reported that breast cancer cells contained phenotypically diverse populations where $CD44^+/CD24^{low}/lineage^-$ population could be highly tumorigenic and form tumors containing various populations of cancer cells. On the other hand, cells with phenotypes diverging from $CD44^+/CD24^{low}/lineage^-$ failed to form tumors even when tens of thousands were injected. Shortly after that, CSCs were also isolated from brain tumors and identified as $CD133^+$ cells, which could produce tumors when injected as 100 cells in the brain of immunodeficient mice. However, injection of 10000-fold more of $CD133^-$ cells failed to grow as tumors (Singh et al. 2003).

Colon CSCs were also identified as $CD133^+$ cells, which were highly tumorigenic and represent only 2.5% of cancer cells (Al-Hajj et al. 2003). CSCs were isolated almost from all types of cancers, and different surface and intercellular markers were reported as CSC markers. Recent advance in this field shows that a panel of markers is better than only one to distinguish CSCs and isolate themselves. Characterization of CSCs by more than one marker gives different populations of cells with different tumorigenicity. For example, pancreatic cancer cells expressing $CD44^+/CD24^+/ESA^+$ showed higher tumorigenicity when compared with

$CD44^+/CD24^+$ or $CD44^+/ESA^+$ cells, while c-$Met^{high}/CD44^+$ cells were recently identified as high tumorigenic pancreatic CSCs (Li et al. 2011). In the published literature, the presence of CSCs have been reported in almost all types of cancers where their existence is being considered as vital for tumor initiation, chemotherapeutic resistance, metastasis, and cancer relapse (Fig. 15.2).

Since the isolation of CSCs mainly depends on their specific surface markers, identification of these markers is a crucial task for CSC research. However, considering the requirements for antibody-based targeting, the lack of CSC-specific surface markers and the low rate of CSC existence in tumor specimens, identification and isolation of CSCs remain as unsurmountable challenge. The complexity and dynamic nature of cancer further render its theranostics a significant challenge. Moreover, the maintenance conditions of CSCs in vitro also need to be optimized where their stemness and differentiation status could be assessed.

Therefore, CSC-relevant research requires novel technology that could handle CSC-based experiments in optimum fashion and mimic their in vivo microenvironment to the best for their optimal maintenance in vitro. The novel in vitro models applying innovative technologies to identify, isolate, or alter normal cells into CSCs are anticipated.

15.3 Utilizing iPSC in Cancer Science

In 2006, the first iPSCs were introduced by Shinya Yamanaka, who used four pluripotency-related transcription factors (Oct4, Sox2, Klf4, and c-Myc) to reprogram mouse fibroblasts into ESCs-like cells (Takahashi and Yamanaka 2006). The publication of Yamanaka's reprogramming protocol paved the way for reversing the terminally differentiated somatic cells by reprogramming, such as fibroblasts, bone marrow cells, skeletal myoblasts, or peripheral blood monocytes, into ESCs-like cells (Ahmed et al. 2011; Buccini et al. 2012). The iPSCs are now considered as surrogate ESCs that have the ability to differentiate to all cell phenotypes of the three germ layers. One of the advantages of iPSCs generation technology is patient-specific and disease-specific iPSCs with wide range of applications in regenerative medicine, drug development, and disease modeling in vitro.

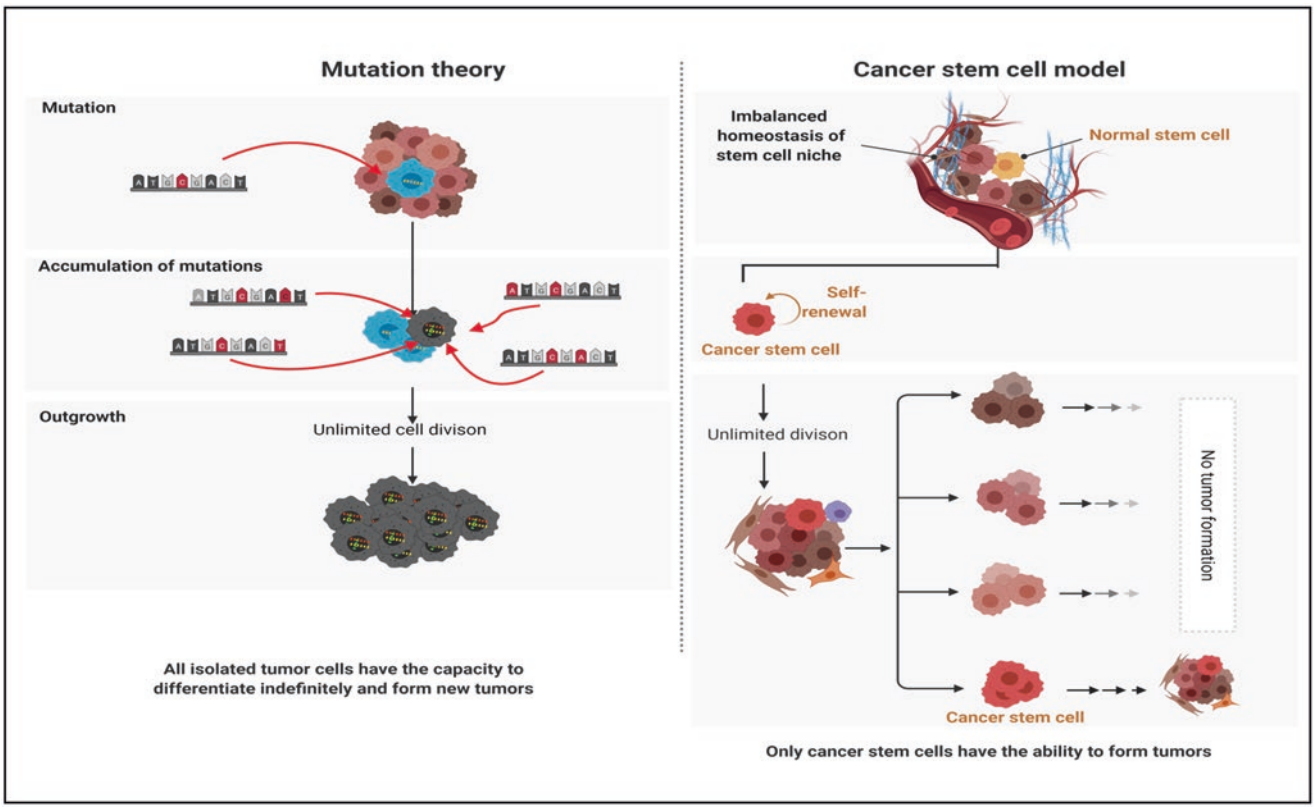

Fig. 15.2 Schematic illustration for the somatic mutation theory and cancer stem cell (CSC) models of tumorigenesis. The somatic mutation theory suggests that the accumulation of multiple mutations or genetic defects transforms cells into cancer cells and acquires unlimited divisions. The cancer stem cell model through epigenetics suggests that the disturbance of homeostasis in stem cell niches leads to their transformation into CSCs. CSCs, with self-renewal and differentiation abilities, form heterogeneous tumors where only CSC subpopulations can initiate tumor formation

iPSCs are similar to ESCs in terms of morphology, pluripotency-related gene expression profiles, epigenetic status, proliferation potential, teratogenicity, and differentiation. However, they are superior to ESCs in terms of availability autologous source and that too without ethical and moral issues. In iPSCs, each cell contains a full set of genomes, and its identity and function depend upon the activation status of genes contained therein. For example, skin cells have activated genes for skin function, while other cell types' specific genes are turned off. Given that iPSCs have pluripotent differentiation potential and infinite proliferation capacity, they usually form teratomas containing cells from the three germ layers but without the metastatic capability. The c-Myc is one of the exogenous reprogramming factors included in the classical quartet of Yamanaka's reprogramming protocol (Omole and Fakoya 2018). However, c-Myc is an oncogene that plays a vital role during embryonic development. c-Myc gets reactivated again after iPSCs generation causing the development of malignant tumors in rare cases. Therefore, it has been dispensed away during the subsequently reported protocols for iPSCs generation. Recently, Liu P et al. selected only two transcription factors (Oct4 and Sox2) of the four classical transcription factors by using CRISPR/Cas gene regulation technology to create iPSCs (Liu et al. 2018).

In succession, the accelerated development of iPSC technology by employing nonintegrating viral vectors, nonviral vectors, or removing the introduced foreign genes via gene knockout has ensured the yields of much safer iPSC (Ibrahim et al. 2016). Meanwhile, some researchers discovered that several chemical compounds were potent in accelerating cellular reprogramming. The process of reprogramming is complex and regulated delicately. Some compounds can significantly improve the reprogramming process's efficiency through activation or inhibition of multiple signaling pathways involved therein. Valproic acid (VPA) and sodium butyrate are histone deacetylase inhibitors found to increase the reprogramming efficiency by more than 100 times and 15−50 times, respectively (Huangfu et al. 2008; Mali et al. 2010). Shi et al. found that the combination of small molecules BIX-01294 and BayK8644 may be combined with only Oct4 and Klf4 for successful reprogramming of mouse embryonic fibroblasts thus indicating that these two small molecules can increase the efficiency and rate of cellular reprogramming or to successfully replace Sox2 in the production of iPSCs (Shi et al. 2008). The combination of SB43142 and PD0325901 could also significantly improve the efficiency of reprogramming. The combination of thiazovivin, an inhibitor of the ROCK pathway, SB43142, an inhibitor of TGF-β receptor, and PD0325901, an inhibitor of the MEK signaling pathway, could increase the efficiency by more than 200 times and shortened the time of reprogramming (Lin et al. 2009). The iPSC technology has become one of the most sought-after topics in stem cell research and helped significant progress in this field.

The iPSC reprogramming technology has also provided new opportunities and insights for cancer research, especially the concept of the existence of CSCs (Fig. 15.3).

Since the discovery of iPSCs technology, scientists have been trying to invest in this technology to create CSCs and study their characteristics. For example, Wong et al. transformed keratinocytes with c-Myc, Ras, and IκB, resulting in the acquisition of CSC phenotypes with high tumorigenicity and similarity with ESCs. They proposed the term "induced cancer stem cells" (iCSCs) as the benefit of reprogramming technology. Exploiting the wide range of differentiation capacity of iPSCs, some scientists could also create patient-derived cancer models to study sequential stages and molecular events of cancer initiation and progression. To this end, either iPSCs may be reprogrammed from normal somatic cells followed by the induction of mutation(s) or disease-specific or patient-derived cells may be reprogrammed to study the role of specific genes in cancer initiation in the context of pluripotency (Wong et al. 2008). Recently, genetically engineered mice-derived cells, in which expression of exogenous reprogramming factors (Oct3/4, Sox2, Klf4, and c-Myc) are controlled by doxycycline (Dox), have been used to study the effects of reprogramming event in vivo. This study showed that transient expression of the reprogramming factors induced by Dox administration resulted in the tumor development in different organs where tumor cells were distinct from teratoma cells and gained the gene expression signature akin to ESCs. The same group also reported that KRAS and TP53 mutations are not sufficient for pancreatic cell transformation, while mutant pancreatic cells transiently reprogrammed by iPSC transcription factors showed characters of early stages of pancreatic cancer development represented by acinar to ductal metaplasia (ADM). Moreover, TP53 cooperates with KRAS and accelerates the induction of pancreatic ductal adenocarcinoma (PDAC) (Shibata et al. 2018).

In the case of prostate tumor development, Zhao et al. found that the deletion of Tgfbr2, and phosphatase and tensin homolog (Pten) genes, increased the reprogramming efficiency of somatic cells by more than fourfold. When mice models were engineered as $Pten^{-}/Tgfbr2^{-}$, the deletion of these two genes promoted cancer growth and its invasiveness besides the induction of pluripotency markers, i.e., Nanog, Sox2, Oct4, and Cripto genes. Moreover, the expression levels of Nanog, Sox2, and Oct4 increased when iPSCs were reprogramed from Pten and TGFβr2 knockout cells (Zhao et al. 2018). In modeling neural cancers, the neural progenitor cells (NPCs) were differentiated from iPSCs, which had P53, Src, and EGFR mutations. These cells exhibited glioma CSC characteristics, which were highly tumorigenic and led to aggressive tumor growth. Different anticancer agents were

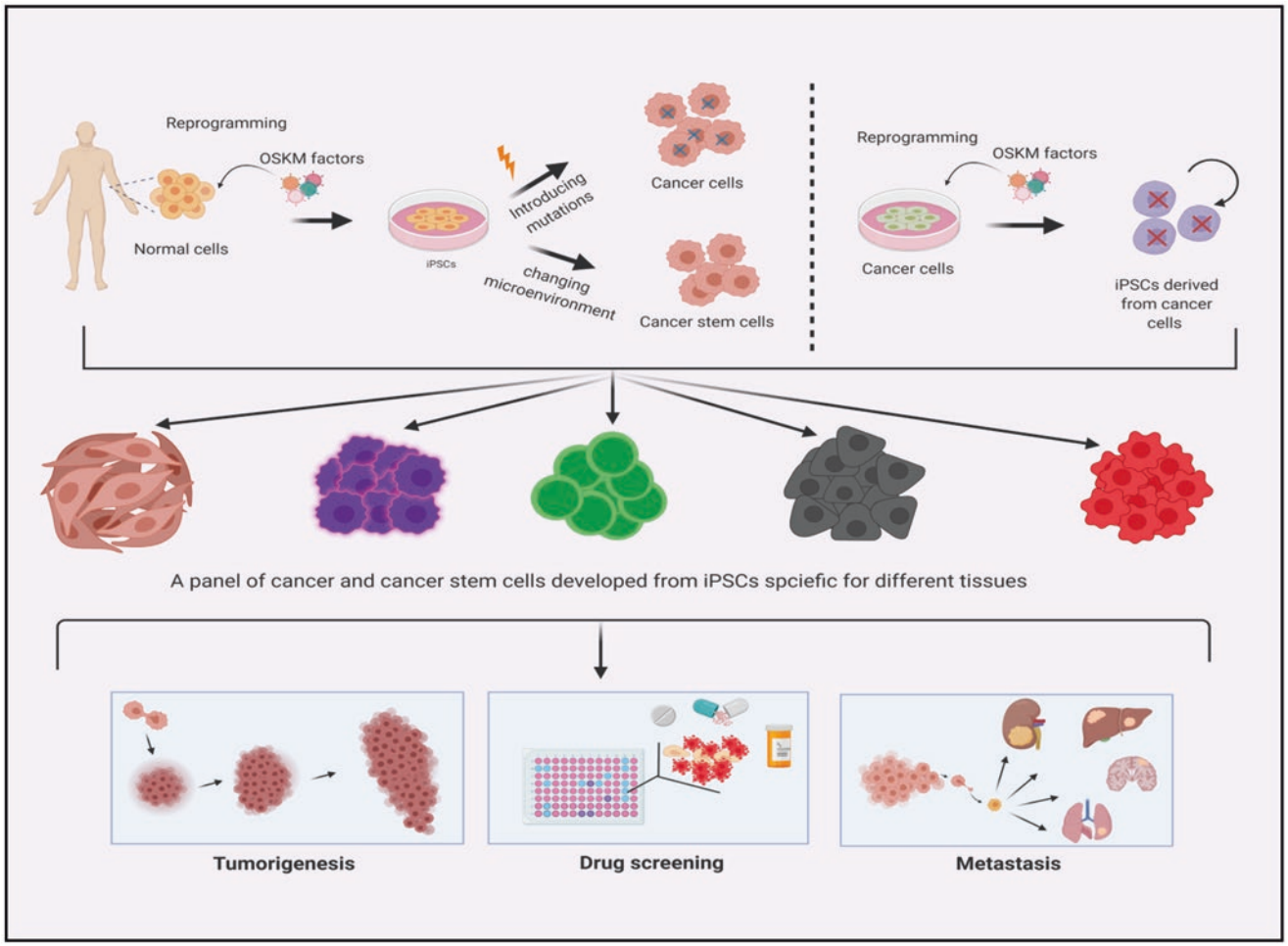

Fig. 15.3 Different approaches to using iPSCs technology in cancer science. Normal cells reprogrammed into iPSCs are being used to create cancer models by introducing mutations or being converted into CSCs by changing its microenvironment. At the same time, cancer cells could be reprogrammed into iPSCs giving iPSCs derived from cancer cells. Collectively, these cells can provide novel models for cancer stem cells or cancer cells deriving from iPSCs. These cells will be available to study tumorigenesis mechanisms, metastasis process, and drug screening

screened on this model to identify drugs targeting glioma CSCs efficiently (Sancho-Martinez et al. 2016) (Fig. 15.3).

In this approach, iPSCs are being used as tools to study tumor progress by introducing mutations in iPSCs or establishing iPSCs from genetically deficient cells. Thus, the effect and role of specific genes and their abnormalities and epigenetic changes in cancer induction and CSCs' maintenance could be assessed. Another more frequently adopted and systematic approach in cancer research is reprogramming of cancer cells into iPSCs to establish iPSCs-derived cell lines from cancer cells. This method helps us understand the nature and identity of cancer cells. In general, reprogramming of cancer cells changes their epigenetics and results in identity changes. If iPSCs derived from reprogramming of cancer cells are injected into immunodeficient mice, they will form teratomas. Melanocytes, pancreatic, and colorectal cancer cells were successfully reprogrammed with different factors to generate iPSCs from the respective cell type. It was interesting to observe that the derivative cells lost their tumorigenicity and chemoresistance characteristics in some cases (Marin Navarro et al. 2018; Czerwinska et al. 2018; Gong et al. 2019). On the contrary, reprogramming of breast cancer cell line MCF-7 cells into iPSCs did not reduce tumorigenicity, rather their tumorigenic properties were increased, resulting in a more aggressive undifferentiated invasive cancer phenotype. The reprogrammed cells were named cancer stem-like cells. Notably, iPSC markers, Oct3/4, Nanog, and SSEA-1 were not upregulated at this time after iPSC induction, unlike usual, but Sox2 was upregulated.

One of the most challenging issues in this method is the low efficiency of reprogramming. This may indicate that only a meager population of cancer cells, less than 1% of cancer cells, is reprogrammed, and the reprogramming cells

does not reflect the nature of cancer cell heterogeneity at the cellular or molecular levels (Chao and Chern 2018). Undeniably, iPSCs share many characters with CSCs, such as self-renewal and differentiation, thus making their investment in cancer science very attractive. At present, the research of iPSCs for cancer is in its infancy and is limited to experimental research. iPSCs reprogrammed from normal cells offer novel methods to generate CSCs without introducing any mutations and foreign genes exploiting the iPSC pluripotency.

During the development of a novel method in our laboratory, we used iPSCs reprogrammed from normal cells to generate CSCs for different cancer types. The conversion of iPSCs into CSCs was based on epigenetic changes and signaling pathway alterations under chronic inflammatory or cancerous microenvironment, exposing iPSCs to a cocktail of growth factors, cytokines, chemokines, and tissue-derived specific factors. The conditioned media (CM) were prepared from cancer cell lines creating such microenvironment, in which iPSCs were cultured for their conversion to CSCs. CM from different cell lines exhibited diverging potentials for conversion (Chen et al. 2012). In our method, we treated the iPSCs with CM prepared from different cancer cell lines. The iPSCs after treatment were named "converted iPSCs" (ciPSCs). This method's novelty was the usage of CM from cancer cell lines to direct the differentiation of iPSCs toward CSCs without any genetic modifications (Fig. 15.4).

To date, we successfully generate CSCs models for lung, pancreatic, breast, and liver cancers. Our first successful report was published in 2012 wherein the conversion of mouse iPSCs (miPSCs) into CSCs was performed using CM from Lewis lung carcinoma (LLC) cell line cells. The miPSCs used in this study were harboring GFP gene under the control of the Nanog promoter. The resulting cells stably expressed GFP in an undifferentiated state corresponding with the Nanog expression but lost its expression once differentiated. miPSCs required leukemia inhibitory factor (LIF) to maintain their stemness in vitro. The cells cultured without LIF underwent differentiation and did not survive. Interestingly, in our experiments, the miPSCs survived in CM without LIF. Cells treated with the CM from LLC cells for 4 weeks were named miPS-LLCcm cells, which kept expressing key markers of stemness and self-renewal such as Nanog, Eras, Rex1, and Cripto. Furthermore, these cells fulfilled the primary criteria to define CSCs by exhibiting sphere-forming ability in low adherent culture conditions and tumorigenicity in Balb/c nude mice (Chen et al. 2012). The pancreatic CSCs were generated from miPSCs following their treatment with CM derived from human pancreatic cancer cell lines, PK8, and KLM-1. In this study, CSCs converted in vitro were enriched via subcutaneous transplantation in nude mice, just as described by the previous studies, followed by transplantation into the pancreas. This orthotropic transplantation led to the enrichment of pancreatic CSCs, which in turn generated tumors imitating pancreatic ductal adenocarcinoma phenotype (PDAC) with liver metastasis. The analysis of RNA sequencing (RNA-seq) data for established CSCs indicated an elevation in the expression of transcription factors specific to pancreatic progenitor cells such as Pdx1, Hes1, Foxa2, Hnf1a, Hnf4a, Pax6, Nr5a2, Rbpj, Rbpgl, MafA, and MafB. PDAC-related hallmarks such as Kras, Krt19, Col8a1, Col1a1, Cxcr4, Muc1, Muc5aC, Mmp2, and Malat1 were also upregulated as well as the most representative pancreatic CSCs-specific markers including CD133, CD24, EpCAM, and CD44 (Calle et al. 2016).

Recently, we demonstrated for the first time that liver CSCs could be generated from iPSCs by culturing iPSCs in the presence of CM of hepatocellular carcinoma cell line (Huh7) cells. As a result, after 4 weeks of culturing miPSCs in the presence of CM, CSCs were induced as miPS-Huh7cm cells, which formed malignant tumors in the liver after 28 days of orthotropic injection into the liver. Primary cells from the malignant tumor of miPS-Huh7cm cells exhibited a similar phenotype to liver CSCs, defined by self-renewal capacity, differentiation potential, and tumorigenicity in vivo. The malignant tumors showed significant expression of the markers mostly common to the liver cancer, such as alpha-fetoprotein (AFP), glypican-3 (GPC3), carcinoembryonic antigen (CEA), and cytokeratin-19 (CK19). The significantly high expression of CD24, CD44, and CD133 was observed in the cells from malignant tumors when compared to miPSCs (Afify et al. 2020).

As we mentioned above, many chemical compounds could change cell epigenetics and assist in the reprogramming process. We assessed the risk of 110 non-mutagenic chemical compounds, most of which are known as inhibitors of cytoplasmic signaling pathways, as potential carcinogens. We treated miPSCs with each compound for 1 week in the presence of a CM of LLC cells. Even a period of 1 week was too short for the CM to convert miPSCs into CSCs. Different compounds showed different potential to accelerate the conversion, where 1 week was enough for induction.

Consequently, PD0325901 (MEK inhibitor), CHIR99021 (GSK-3β inhibitor), and Dasatinib (Abl, Src, and c-Kit inhibitor) were found to confer miPSCs into CSC phenotype in 1 week. The survived converted cells exhibited stemness markers expression, spheroid formation ability, and tumorigenesis in Balb/c nude mice (Du et al. 2020). Finally, several different protocols now exist, investing the iPSCs in creating models for cancer study. These protocols, however, differ from each other depending upon the purpose of the research and the perspective of the researchers. While the primary propose still remains the uncovering and understanding of tumorigenesis mechanisms and exploration of new targets for treatment or new therapeutic strategies, these models are expected to have great impact on cancer prevention, diagnosis, and drug development.

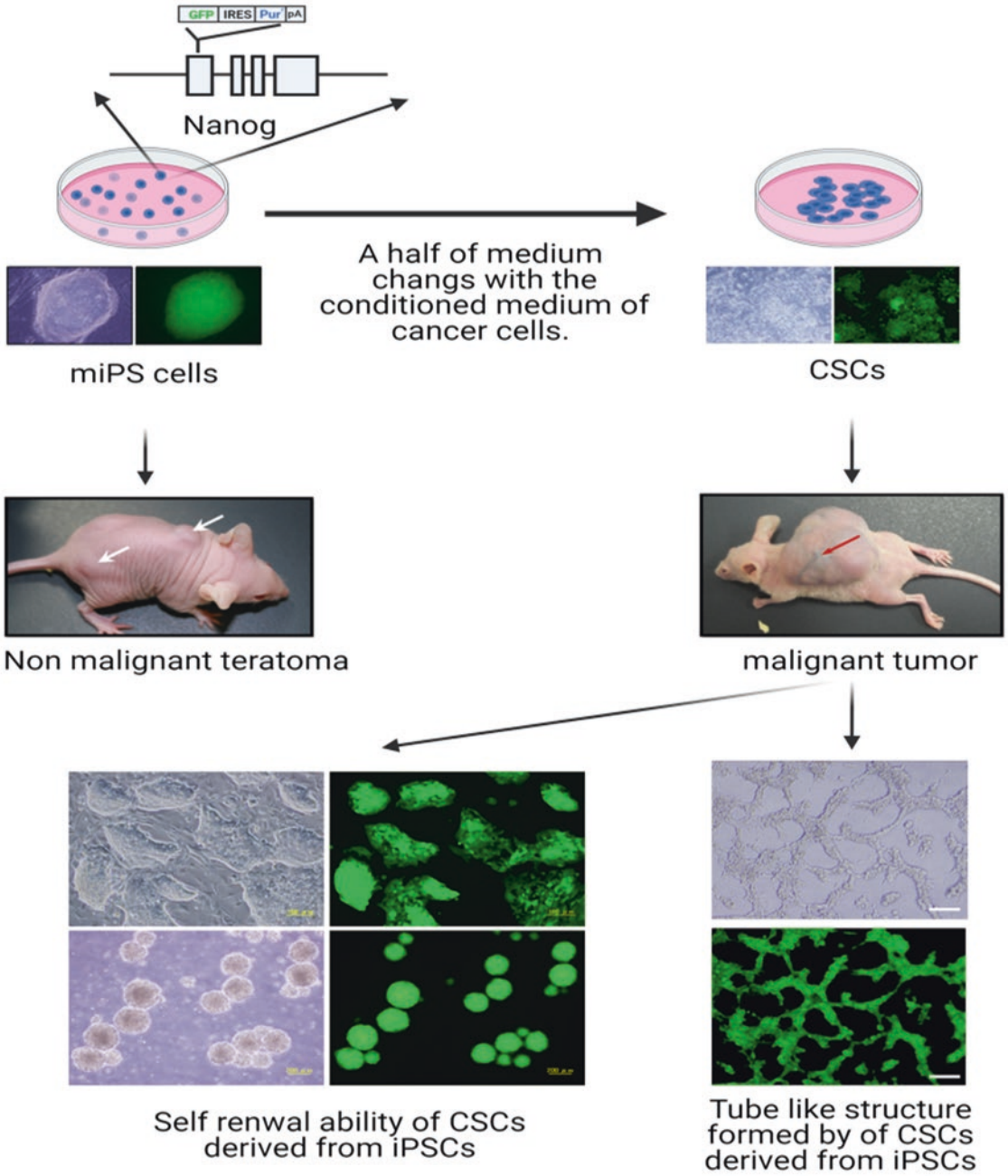

Fig. 15.4 Conversion of iPSCs into CSCs using conditioned media from cancer cells. The green fluorescent protein (GFP) and puromycin resistance genes were introduced into miPSCs under the Nanog promoter. The miPSCs were cultured in media containing 50% conditioned media prepared from cancer cells. One-month treatment converts miPSCs into CSCs. Injection of miPSCs into nude mice resulted in the formation of teratoma; however, injection of CSCs converted from miPSCs produced malignant tumors. Isolated CSCs from primary tumors expressed GFP and could form spheroids in low adherent culture conditions which indicate self-renewal ability. CSCs also demonstrated the ability to form tube-like structures and differentiate into endothelial cells. (A part of this figure was taken from (Chen et al. 2012))

15.4 Investigation of Tumor Initiation Mechanisms with iPSCs

The first stage of cancer development is usually very slow and hence prolonged and stepwise. Besides, it takes for the developing cancer many years to be noticeable. Recently, many changes in the cancer concept have been proposed, and scientists became more flexible in considering different ideas of tumorigenesis. Great efforts have been made to identify the origin of CSCs and explore the potential mechanisms of cancer initiation. During the last half century, research in stem cell biology accumulated information and proposed

that CSCs could be developed from stem cells residing in each tissue. The proposed origin of CSCs from stem cells was explained by either mutation theory or inflammation and epigenetic concepts. Thus, stem cells have attracted cancer researchers to uncover the development process of CSCs and their role in cancer diagnosis, metastasis, and as a novel target in cancer therapeutics.

Recent studies show that iPSCs could be a useful source to uncover cancer initiation and progression (Fig. 15.3). For example, iPSCs reprogrammed from PDAC cells were used to study pancreatic cancer initiation. These iPSCs formed the early stage of invasive ductal adenocarcinoma, pancreatic intraepithelial neoplasia (PanIN), and then developed into invasive ductal adenocarcinoma after injection into immunodeficient mice. Moreover, cells derived from these iPSCs tumors had the same phenotype of human pancreatic adenocarcinoma. This study showed the ability of iPSCs reprogrammed from cancer cells to capture different stages of cancer progression when they are differentiated back into the original phenotype (Kim et al. 2013). The iPSCs were also generated from patients with myelodysplastic syndromes, a bone marrow disorder and a type of blood cancer. In this case, the iPSCs were reprogrammed from both normal cells and cells with chromosome 7q deletion. The comparison between these two types of iPSCs showed a variation in the differentiation ability. The chromosome 7q deletion impairs the iPSCs differentiation potential into hematopoietic cells, which signifies the role of this deletion in immature cell production in the myelodysplastic syndrome (Kotini et al. 2015, 2017).

The pediatric myeloproliferative disorder, juvenile myelomonocytic leukemia (JMML), mainly affects children that make specimens hard to obtain. The availability of JMML specimens for cancer progression studies and drug screening is limited. The iPSCs reprogrammed from cells of those patients are valuable tools to create disease models. Gandre-Babbe et al. developed iPSCs from juvenile myelomonocytic leukemia patients and used them for drug screening and study clonal and differentiation potentials (Gandre-Babbe et al. 2013). Also, iPSCs could be reprogrammed from mutant noncancer cells taken from volunteers with high risk for specific types of cancers, such as women with germline mutations in BRCA1, which is an increased risk for breast cancer. Griscelli et al. successfully generated iPSCs from blood samples taken from a triple-negative breast cancer patient with BRCA1 mutations (Griscelli et al. 2017). In colon cancer, APC mutations are linked to a high risk of cancer incidence. To understand the relationship between APC mutation and colon cancer induction, Sommer et al. established iPSCs from APC mutant cells and normal cells and then compared them to each other. When differentiated into the intestinal progeny, APC mutations dysregulated signaling pathways and changed lipid metabolism causing abnormalities and resulting in the cell phenotype change. Modeling colon cancer with iPSCs could give insights into the earlier stages of tumorigenesis in the colon mediated by APC (Sommer et al. 2018).

CSCs converted from iPSCs are highly useful to study epigenetic alterations affecting normal stem cells to transform themselves into CSCs. In attempts to find epigenetic changes through the conversion of iPSCs into CSCs, we evaluated the levels of methylation in the genome during CSCs development from iPSCs under the tumor microenvironment (Oo et al. 2018). The methylation status of CpG-islands in CSCs was compared with that in iPSCs. The differentially methylated regions (DMRs) showed that hypomethylation significantly appeared in CSCs when compared to hypermethylation. Furthermore, analysis of the hypomethylated genes by the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool coupled with the KEGG pathway database revealed that several cancer-related pathways were enriched in CSCs derived from iPSCs. Among the nominated genes, high expression of modules such as pik3ca, pik3cb, pik3r1, and pik3r5 genes in the PI3K-Akt signaling pathway was detected. Accordingly, Akt phosphorylation was found to be increased in the obtained CSCs. Therefore, the activation of the PI3K-Akt signaling pathway was involved in the conversion of iPSCs into CSCs with high malignancy and metastatic potential. In a similar way, our previous study demonstrated that different chemical compounds such as Dasatinib, PD0325901, and CHIR99021 accelerated the conversion of iPSCs into CSCs in the presence of CM from LLC cells. Taking into account the signaling pathways inhibited by these compounds, the inhibition of Erk1/2, tyrosine kinase, and/or Gsk-3β was indirectly involved in the enhancement of the PI3K-Akt signaling pathway, resulting in the sustained stemness properties and enhancing the malignant transformation of iPSCs (Du et al. 2020).

We also showed that CSCs, which were derived from iPSCs under pancreatic cancerous microenvironment derived from the CM of PK8 cells, exhibited high expression of ErbB2 and ErbB3 genes and those related to PI3K pathway. Moreover, the inhibition of ErbB2 in iPSCs by lapatinib arrested cell proliferation and impaired the conversion process. This study shows the potential role of ErbB2/ErbB3 heterodimer and its related pathway, PI3K, in CSCs generation and could lead to potentially new options for cancer treatment and prevention (Hassan and Seno 2020a).

15.5 Using iPSCs-Derived CSCs to Study Cancer Microenvironment and Heterogeneity

Tumors have heterogeneous structures that contain many different phenotypes of cells. The tumor microenvironment comprises of different cell types such as fibroblasts, endothe-

lial cells, and immune cells. The tumor microenvironment raises many questions about the mechanisms and interactions controlling tumor heterogeneity. The cellular plasticity which may result in tumor heterogeneity was explained by the concept of CSCs. The cellular plasticity of CSC results from the self-renewal and differentiation potential. Therefore, the development of CSC models will assist in understanding the cancer microenvironment and heterogeneity. CSCs developed from miPSCs were shown to have the ability to differentiate into vascular endothelial cells and contribute to the tumor angiogenesis process (Matsuda et al. 2014). In this study, CSCs were confirmed to develop vascular tube-like structures when cultured on Matrigel (Fig. 15.4). The in vitro tube formation capacity in CSCs showed a dependency on autocrine effects of the angiogenic factors expressed from CSCs such as vascular endothelial growth factor A (VEGF-A) and basic fibroblast growth factor (bFGF) during in vitro tube formation assay. These findings are the first to report in literature and provide insights into the ability of CSCs to generate a self-sustaining niche in the presence of tumor-derived soluble and/or paracrine factors.

In a comprehensive in vivo study, CSC developed from miPSCs displayed critical role in the recruitment of host endothelial vessels into a tumor and the differentiation into endothelial linage, including vasculogenic mimicry (Prieto-Vila et al. 2016). These results show that CSCs have a critical role in tumor vasculature, which could be a good target for cancer therapies in the future. In the same context, we demonstrated that miPSCs-derived CSCs could establish their niche by differentiating into fibroblasts. We concluded that CSCs were the potential origin of the cancer-associated fibroblasts (CAFs), an essential cell type in the stromal compartment of the tumor microenvironment. In this study, the CSCs converted from miPSCs were transplanted into the mammary fat pad of nude mice, and the resulting tumor primary cells expressed CSC-specific markers. CSCs exhibited the ability to differentiate into CAF-like phenotype thus suggesting that they had differentiated into subpopulations of cells that support CSC self-renewal. This was confirmed by evaluating the expression of CAF markers such as smooth muscle actin (α-SMA), fibroblast-specific α-protein (FSP1), TGFβ1, stromal-derived factor-1 (CXCL12), and platelet-derived growth factor (PDGFα), collagen type I alpha 1 (Col1α1) and vimentin. These markers were upregulated in the differentiated myofibroblast-like cells derived from CSC spheroids. These results confirmed that CSCs could be the source of CAFs in the tumor microenvironment (Nair et al. 2017).

In the tumor microenvironment, different hematopoietic cells play critical functions in tumor growth and progression (Gajewski et al. 2013; Hassan and Seno 2020b; Pages et al. 2005; Salama et al. 2009). We also described that adherent CSCs could give hematopoietic cells. In our recent study, we observed that the non-adherent cells (NACs) originated from adherent CSCs and expressed different hematopoietic cell markers, such as CD10, CD34, CD38, c-kit, and Runx1. Also, NACs could home into the bone marrow as well as hematopoietic progenitor cells after injection into the tail vein of busulfan-conditioned nude mice. Another study showed that CSCs deriving from human iPSCs had differentiation capacity into macrophages in vivo. These studies opened the door for further investigation of the origin of immune cells in the tumor microenvironment and the role of CSCs in this field (Hassan et al. 2019; Osman et al. 2020).

Unfortunately, 2D culture models limit cells in one environment providing the only cell-to-cell interaction and failing to represent the human body's complexity. In this context, 3D organoids are necessary to mimic the heterogeneity of different cellular niches. Since cancer has been described as heterogeneous tissue, 3D models mimicking tumor tissue, including the microenvironment in its natural habitat, are required. These models could provide more practical and relevant tools than 2D models during the early stages of anticancer drug screening in vitro. Moreover, the availability of the 3D models will minimize the dependency on animal experiments. The 3D models of cancer using iPSCs provide novel tools for cancer research. More recently, cancer organoid technology employing iPSCs has been described with differentiation and gene editing methods such as CRISPR/Cas. In a recent study, iPSCs were reprogramed from somatic c-Met mutant cells taken from a patient with type 1 papillary renal cell carcinoma (PRCC) and then differentiated into kidney cell progenitors in a 3D environment. When differentiated, established organoids expressed PRCC markers indicating that the cancer initiation process was triggered (Hwang et al. 2019). This combination of different technology presents new opportunities to study human cancers by developing wide scale in vitro models (Papapetrou 2016).

15.6 Drug Screening, Precision Medicine, and New Treatment Strategies

Drug resistance is one of the biggest problems in the cancer field, and many researchers are working to develop effective anticancer drugs. Despite the considerable advancements in the cancer science, there are still many patients suffering from untreatable cancers. The drug resistance, complexity of cancer pathology, and the presence of CSCs which have been implicated in cancer progression and relapse are believed to be responsible for the poor prognosis and overall low survival rate in cancer patients (Kim 2015).

Although many anticancer drugs and treatments for patients currently exist, the effective treatment strategy must be determined depending on each type of cancer. Moreover, cancer is hypothesized as a patient-specific disease that sub-

stantiates the heterogeneity of cancer existing between patients. Genetic variability and epigenetic factors are considered responsible for this heterogeneity (Guo et al. 2019). Therefore, the treatment strategy should be determined for each patient, depending on these factors. Precision medicine or personalized medicine selects a treatment strategy based on the genetic information of cancer patients. The recent advances in the genetic analysis technologies have accelerated precision oncology research. In this context, iPSC is an attractive tool providing cancer models specific to each individual. When combined with new genetic technology, one advantage of iPSC technology is the ability to give the models of cancer and CSC for individuals who have risks of cancer even before cancer develops. The prediction of effective treatment for different types of cancer could be possible with these models. The CSCs derived from the patient's iPSCs will enable screening other treatments and selecting the appropriate one depending on personal genetic and epigenetics profiles. Such predictive approaches could also take advantage of genomic, transcriptomic, proteomic, and metabolomic analyzing tools (Kim 2015; Papapetrou 2016). CSCs are considered resistant to drugs because of their stem cell-like properties such as dormancy, drug export, and high survival capacity and their niches. Therefore, current models for drug screening should consider CSC niche and tumor heterogeneity.

CSCs integrated with advanced 3D-culture technologies could form a useful source for drug screening. Since CSCs are hard to be obtained and maintained in culture, CSCs generated from iPSCs could substitute those from patients or their patient-derived xenografts (PDX) models as a renewable source. CSCs construct their niches by differentiating into cancer cells or cancer-associated cells, as mentioned above, producing heterogeneity in the tumor microenvironment. In a recent study, different types of normal neural cells such as neurons, astrocytes, and glial cells were derived from iPSCs and cultured in a 3D environment with glioblastoma tumor cells. This 3D model was used as an anti-glioblastoma drug screening platform and suggested as a tool with the availability to execute several assays simultaneously in the same condition. We also did a drug screening test on CSC deriving from miPSCs using around 200 anticancer drugs from the screening committee of anticancer drugs (SCADS) library. We showed that daunorubicin, a topoisomerase II inhibitor, can eliminate CSCs in a mechanism associated with caspase pathway activation and P53 accumulation (Seno et al. 2019). We also showed that the combination of paclitaxel and sorafenib could be very effective in suppressing CSC's self-renewal ability when tested on CSCs derived from miPSCs. This combination showed a synergistic effect (Nawara et al. 2020).

The iPSC-derived cells can be used to evaluate the toxicity of anticancer drugs toward normal cells. For instance, iPSC-derived cardiomyocytes are used to assess anticancer drugs cardiotoxicity. Assessment of the anticancer drugs on function and morphology is more flexible with iPSC-derived cardiomyocytes than with patient-derived cardiomyocytes (Schwach et al. 2020). Neuronal cells derived from iPSCs were also used to investigate the chemotherapy-induced peripheral neuropathy, which occurs after cancer treatment. Wheeler et al. showed that the effects of neurotoxic drugs differed between patients. This study showed that sensitivity to paclitaxel increased in neurons, while the expression of tubulin beta 2A class IIa (*TUBB2A*) decreased. Therefore, iPSC-derived cells could be more suitable than cell lines to assess drug's neurotoxic side effects, which are different between patients (Rana et al. 2017).

The expression of surface proteins in iPSCs was suggested to be similar to cancer cells. This similarity drove to another idea to use iPSCs as a cancer vaccine. In a recent study, Kooreman et al. prepared iPSCs from mice, impaired their proliferation by irradiation, and injected them into same mice to vaccinate. Then, breast cancer cells were injected into the vaccinated mice with iPSCs. The cells developed tumors, began to shrink, and disappeared compared with those injected into non-vaccinated mice, wherein the newly formed tumors continued to grow. Moreover, T-cells from mice injected with iPSCs were able to suppress cancer and teratoma growth in other unvaccinated mice. This suggests that T-cells activated by iPSCs injection became able to recognize epitopes shared between iPSC and cancer cells (Kooreman et al. 2018). The CSCs derived from iPSCs could also serve as much more active vaccines since the deriving CSCs from iPSCs could reveal other epitopes specific to CSCs. Overall, iPSCs and their derivative cells and models show a wide range of potential applications in drug screening and developing new cancer prevention and treatment strategies.

15.7 Current Challenges and Future Perspectives

The iPSCs provide patient-based models as a novel tool in the bioscience field. The application of iPSCs in cancer science is still relatively new and requires more effort to shape their usage in cancer research. The unique characters of iPSCs make them ideal tools to study tumor initiation and CSCs. On the other hand, the shared characteristics between iPSCs and CSCs bring new insights into the investment of iPSCs in the oncology field.

Subgroups of CSCs are classified depending on specific surface markers showing the different ability of tumor initiation in animal models. The tumorigenicity of CSCs proved to vary between different subpopulations of CSCs. The iPSCs could serve as a starting point to understand the concept of

plasticity in CSCs. Cancer models from iPSCs offer new opportunities to investigate cancer heterogeneity and tumor microenvironment. The map of interactions between CSCs and tumor microenvironment could be illustrated in the future by the use of different approaches such as genetic engineering, iPSCs-derived cells and 3D cell culture models.

Though it has been almost two decades since CSCs were first isolated, a lot of information about regulation mechanisms must be clarified. The comparison of CSCs induced from iPSC with those derived from patients could reveal some novel drug targets, and the origin and fate of CSCs in disrupted tissue microenvironments. In the new era of basic cancer research and precision oncology, iPSCs deriving from cancer patients or healthy individuals will expand our knowledge about cancer, replacing the need for animal experiments in some stages of drug development and accelerating cancer research. The optimized protocols will be necessary to develop iPSC-derived cancer or CSC models that can reflect cancer's heterogeneity and nature. These cells and models will be available for drug screening and deciding treatment strategy. Therefore, interdisciplinary approaches combining cancer researchers, bioinformatic specialists, bioengineers, and drug companies' efforts are needed to make breakthroughs in this direction.

In the end, the iPSC-derived CSCs could be created for a wide range of cancer types and for each individual enabling the collection of big data that reflect the genetic and epigenetic specificity of each individual. This will predict cancer incidence risk, prevention approaches, and personalized drugs and treatment strategies. This could enhance survival rates and decrease the suffering of cancer patients with minimized side effects of cancer treatments. The iPSCs, as a new tool in cancer research, could open the door of different perspectives to be investigated, challenging the old believed concepts about cancer origin and progression.

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References

Afify SM, Sanchez Calle A, Hassan G, Kumon K, Nawara HM, Zahra MH, Mansour HM, Khayrani AC, Alam MJ, Du J, Seno A, Iwasaki Y, Seno M (2020) A novel model of liver cancer stem cells developed from induced pluripotent stem cells. Br J Cancer 122:1378–1390

Ahmed RPH, Haider HK, Buccini S, Li L, Jiang S, Ashraf M (2011) Reprogramming of skeletal myoblasts for induction of pluripotency for tumor-free cardiomyogenesis in the infarcted heart. Circ Res 109(1):60–70

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100:3983–3988

Androutsos G, Karamanou M (2009) Bernard Peyrilhe (1737–1804) and the first experimental transmission of cancer. J BUON 14:731–733

Anttila S, Boffetta P (2014) Occupational cancers, 1st edn. Springer, London

Baylin SB, Jones PA (2016) Epigenetic determinants of Cancer. Cold Spring Harb Perspect Biol 8

Ben-David U, Beroukhim R, Golub TR (2019) Genomic evolution of cancer models: perils and opportunities. Nat Rev Cancer 19:97–109

Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737

Brucher BL, Jamall IS (2014a) Cell-cell communication in the tumor microenvironment, carcinogenesis, and anticancer treatment. Cell Physiol Biochem 34:213–243

Brucher BL, Jamall IS (2014b) Epistemology of the origin of cancer: a new paradigm. BMC Cancer 14:331

Brucher BL, Jamall IS (2016) Somatic mutation theory – why it's wrong for Most cancers. Cell Physiol Biochem 38:1663–1680

Brücher BLDM, Jamall IS (2019) Chronic inflammation evoked by pathogenic stimulus during carcinogenesis. 4open 2:8

Buccini S, Haider HK, Ahmed RPH, Jiang S, Ashraf M (2012) Cardiac progenitors derived from reprogrammed mesenchymal stem cells contribute to angiomyogenic repair of the infarcted heart. Basic Res Cardiol 107(6):301

Buchholz TA, Weil MM, Story MD, Strom EA, Brock WA, Mcneese MD (1999) Tumor suppressor genes and breast cancer. Radiat Oncol Investig 7:55–65

Calle AS, Nair N, Oo AK, Prieto-Vila M, Koga M, Khayrani AC, Hussein M, Hurley L, Vaidyanath A, Seno A, Iwasaki Y, Calle M, Kasai T, Seno M (2016) A new PDAC mouse model originated from iPSCs-converted pancreatic cancer stem cells (CSCcm). Am J Cancer Res 6:2799–2815

Capp JP (2019) Cancer stem cells: from historical roots to a new perspective. J Oncol 2019:5189232

Chao HM, Chern E (2018) Patient-derived induced pluripotent stem cells for models of cancer and cancer stem cell research. J Formos Med Assoc 117:1046–1057

Chen L, Kasai T, Li Y, Sugii Y, Jin G, Okada M, Vaidyanath A, Mizutani A, Satoh A, Kudoh T, Hendrix MJ, Salomon DS, Fu L, Seno M (2012) A model of cancer stem cells derived from mouse induced pluripotent stem cells. PLoS One 7:e33544

Czerwinska P, Mazurek S, Wiznerowicz M (2018) Application of induced pluripotency in cancer studies. Rep Pract Oncol Radiother 23:207–214

Di Lonardo A, Nasi S, Pulciani S (2015) Cancer: we should not forget the past. J Cancer 6:29–39

Du J, Xu Y, Sasada S, Oo AKK, Hassan G, Mahmud H, Khayrani AC, Alam MJ, Kumon K, Uesaki R, Afify SM, Mansour HM, Nair N, Zahra MH, Seno A, Okada N, Chen L, Yan T, Seno M (2020) Signaling inhibitors accelerate the conversion of mouse iPS cells into Cancer stem cells in the tumor microenvironment. Sci Rep 10:9955

Feinberg AP, Vogelstein B (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301:89–92

Fisher JC (1958) Multiple-mutation theory of carcinogenesis. Nature 181:651–652

Gajewski TF, Schreiber H, Fu YX (2013) Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol 14:1014–1022

Gandre-Babbe S, Paluru P, Aribeana C, Chou ST, Bresolin S, Lu L, Sullivan SK, Tasian SK, Weng J, Favre H, Choi JK, French DL, Loh ML, Weiss MJ (2013) Patient-derived induced pluripotent stem cells recapitulate hematopoietic abnormalities of juvenile myelomonocytic leukemia. Blood 121:4925–4929

Golzari SE, Khan ZH, Ghabili K, Hosseinzadeh H, Soleimanpour H, Azarfarin R, Mahmoodpoor A, Aslanabadi S, Ansarin K (2013)

2013. Contributions of medieval Islamic physicians to the history of tracheostomy. Anesth Analg 116:1123–1132
Gong L, Yan Q, Zhang Y, Fang X, Liu B, Guan X (2019) Cancer cell reprogramming: a promising therapy converting malignancy to benignity. Cancer Commun (Lond) 39:48
Griscelli F, Oudrhiri N, Feraud O, Divers D, Portier L, Turhan AG, Bennaceur Griscelli A (2017) Generation of induced pluripotent stem cell (iPSC) line from a patient with triple negative breast cancer with hereditary exon 17 deletion of BRCA1 gene. Stem Cell Res 24:135–138
Guo M, Peng Y, Gao A, Du C, Herman JG (2019) Epigenetic heterogeneity in cancer. Biomark Res 7:23
Hajdu SI (2011) A note from history: landmarks in history of cancer, part 1. Cancer 117:1097–1102
Hajdu SI (2012) A note from history: landmarks in history of cancer, part 4. Cancer 118:4914–4928
Hassan G, Seno M (2020a) Abstract PO-037: the conversion of induced pluripotent stem cells into cancer stem cells under pancreatic cancer microenvironment is inhibiting by lapatinib. Cancer Res 80:PO-037-PO-037
Hassan G, Seno M (2020b) Blood and Cancer: Cancer stem cells as origin of hematopoietic cells in solid tumor microenvironments. Cell 9
Hassan G, Afify SM, Nair N, Kumon K, Osman A, Du J, Mansour H, Abu Quora HA, Nawara HM, Satoh A, Zahra MH, Okada N, Seno A, Seno M (2019) Hematopoietic cells derived from Cancer stem cells generated from mouse induced pluripotent stem cells. Cancers (Basel) 12:82
Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 26:795–797
Hwang JW, Desterke C, Feraud O, Richard S, Ferlicot S, Verkarre V, Patard JJ, Loisel-Duwattez J, Foudi A, Griscelli F, Bennaceur-Griscelli A, Turhan AG (2019) iPSC-derived Embryoid bodies as models of c-met-mutated hereditary papillary renal cell carcinoma. Int J Mol Sci 20:4867
Ibrahim AY, Mehdi Q, Abbas AO, Alashkar A, Haider HK (2016) Induced pluripotent stem cells: next generation cells for tissue regeneration. JBiSE 9(4):226–244
Kim JJ (2015) Applications of iPSCs in Cancer research. Biomark Insights 10:125–131
Kim J, Hoffman JP, Alpaugh RK, Rhim AD, Reichert M, Stanger BZ, Furth EE, Sepulveda AR, Yuan CX, Won KJ, Donahue G, Sands J, Gumbs AA, Zaret KS (2013) An iPSC line from human pancreatic ductal adenocarcinoma undergoes early to invasive stages of pancreatic cancer progression. Cell Rep 3:2088–2099
Kooreman NG, Kim Y, De Almeida PE, Termglinchan V, Diecke S, Shao NY, Wei TT, Yi H, Dey D, Nelakanti R, Brouwer TP, Paik DT, Sagiv-Barfi I, Han A, Quax PHA, Hamming JF, Levy R, Davis MM, Wu JC (2018) Autologous iPSC-based vaccines elicit anti-tumor responses in vivo. Cell Stem Cell 22:501–513. e7
Kotini AG, Chang CJ, Boussaad I, Delrow JJ, Dolezal EK, Nagulapally AB, Perna F, Fishbein GA, Klimek VM, Hawkins RD, Huangfu D, Murry CE, Graubert T, Nimer SD, Papapetrou EP (2015) Functional analysis of a chromosomal deletion associated with myelodysplastic syndromes using isogenic human induced pluripotent stem cells. Nat Biotechnol 33:646–655
Kotini AG, Chang CJ, Chow A, Yuan H, Ho TC, Wang T, Vora S, Solovyov A, Husser C, Olszewska M, Teruya-Feldstein J, Perumal D, Klimek VM, Spyridonidis A, Rampal RK, Silverman L, Reddy EP, Papaemmanuil E, Parekh S, Greenbaum BD, Leslie CS, Kharas MG, Papapetrou EP (2017) Stage-specific human induced pluripotent stem cells map the progression of myeloid transformation to transplantable leukemia. Cell Stem Cell 20:315–328. e7
Li C, Wu JJ, Hynes M, Dosch J, Sarkar B, Welling TH, Pasca Di Magliano M, Simeone DM (2011) C-met is a marker of pancreatic cancer stem cells and therapeutic target. Gastroenterology 141:2218–2227. e5
Liberti MV, Locasale JW (2016) The Warburg effect: how does it benefit Cancer cells? Trends Biochem Sci 41:211–218
Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, Abujarour R, Lin X, Hahm HS, Hao E, Hayek A, Ding S (2009) A chemical platform for improved induction of human iPSCs. Nat Methods 6:805–808
Liu P, Chen M, Liu Y, Qi LS, Ding S (2018) 2018. CRISPR-based chromatin remodeling of the endogenous Oct4 or Sox2 locus enables reprogramming to pluripotency. Cell Stem Cell 22:252–261. e4
Mali P, Chou BK, Yen J, Ye Z, Zou J, Dowey S, Brodsky RA, Ohm JE, Yu W, Baylin SB, Yusa K, Bradley A, Meyers DJ, Mukherjee C, Cole PA, Cheng L (2010) Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. Stem Cells 28:713–720
Manchester K (1997) The quest by three giants of science for an understanding of cancer. Endeavour 21:72–76
Marin Navarro A, Susanto E, Falk A, Wilhelm M (2018) Modeling cancer using patient-derived induced pluripotent stem cells to understand development of childhood malignancies. Cell Death Discov 4:7
Matsuda S, Yan T, Mizutani A, Sota T, Hiramoto Y, Prieto-Vila M, Chen L, Satoh A, Kudoh T, Kasai T, Murakami H, Fu L, Salomon DS, Seno M (2014) Cancer stem cells maintain a hierarchy of differentiation by creating their niche. Int J Cancer 135:27–36
Miller G, Stebbing J (2018) Thirty years of oncogene. Oncogene 37:553–554
Nair N, Calle AS, Zahra MH, Prieto-Vila M, Oo AKK, Hurley L, Vaidyanath A, Seno A, Masuda J, Iwasaki Y, Tanaka H, Kasai T, Seno M (2017) A cancer stem cell model as the point of origin of cancer-associated fibroblasts in tumor microenvironment. Sci Rep 7:6838
Nawara HM, Hassan G, Zahra MH, Atallah MN, Mansour H, Abu Quora HA, Alam MJ, Osman A, Kakuta H, Hamada H, Seno A, Seno M (2020) Paclitaxel and Sorafenib: the effective combination of suppressing the self-renewal of Cancer stem cells. Cancers (Basel) 12:1360
Neff EP (2016) Models matter in metastasis. Lab Anim (NY) 46:3
Omole AE, Fakoya AOJ (2018) Ten years of progress and promise of induced pluripotent stem cells: historical origins, characteristics, mechanisms, limitations, and potential applications. PeerJ 6:e4370
Oo AKK, Calle AS, Nair N, Mahmud H, Vaidyanath A, Yamauchi J, Khayrani AC, Du J, Alam MJ, Seno A, Mizutani A, Murakami H, Iwasaki Y, Chen L, Kasai T, Seno M (2018) Up-regulation of PI 3-kinases and the activation of PI3K-Akt signaling pathway in Cancer stem-like cells through DNA Hypomethylation mediated by the Cancer microenvironment. Transl Oncol 11:653–663
Osman A, Oze M, Afify SM, Hassan G, El-ghlban S, Nawara HM, Fu X, Zahra MH, Seno A, Winer I, Salomon DS, Seno M (2020) Tumor-associated macrophages derived from cancer stem cells. Acta Histochem 122:151628
Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J (2005) Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 353:2654–2666
Papapetrou EP (2016) Patient-derived induced pluripotent stem cells in cancer research and precision oncology. Nat Med 22:1392–1401
Prieto-Vila M, Yan T, Calle AS, Nair N, Hurley L, Kasai T, Kakuta H, Masuda J, Murakami H, Mizutani A, Seno M (2016) iPSC-derived cancer stem cells provide a model of tumor vasculature. Am J Cancer Res 6:1906–1921
Rana P, Luerman G, Hess D, Rubitski E, Adkins K, Somps C (2017) Utilization of iPSC-derived human neurons for high-throughput

drug-induced peripheral neuropathy screening. Toxicol In Vitro 45:111–118

Ribatti D (2008) Judah Folkman, a pioneer in the study of angiogenesis. Angiogenesis 11:3–10

Ribatti D (2018) An historical note on the cell theory. Exp Cell Res 364:1–4

Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, Platell C, Iacopetta B (2009) Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 27:186–192

Sancho-Martinez I, Nivet E, Xia Y, Hishida T, Aguirre A, Ocampo A, Ma L, Morey R, Krause MN, Zembrzycki A, Ansorge O, Vazquez-Ferrer E, Dubova I, Reddy P, Lam D, Hishida Y, Wu MZ, Esteban CR, O'leary D, Wahl GM, Verma IM, Laurent LC, Izpisua Belmonte JC (2016) Establishment of human iPSC-based models for the study and targeting of glioma initiating cells. Nat Commun 7:10743

Schwach V, Slaats RH, Passier R (2020) Human pluripotent stem cell-derived cardiomyocytes for assessment of anticancer drug-induced cardiotoxicity. Front Cardiovasc Med 7:50

Seno A, Mizutani A, Aizawa K, Onoue R, Masuda J, Ochi N, Taniguchi S, Sota T, Hiramoto Y, Michiue T, Nair N, Seno M (2019) Daunorubicin can eliminate iPS-derived cancer stem cells via ICAD/CAD-independent DNA fragmentation. Cancer Drug Resist 2:335–350

Shi Y, Desponts C, Do JT, Hahm HS, Scholer HR, Ding S (2008) Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell 3:568–574

Shibata H, Komura S, Yamada Y, Sankoda N, Tanaka A, Ukai T, Kabata M, Sakurai S, Kuze B, Woltjen K, Haga H, Ito Y, Kawaguchi Y, Yamamoto T, Yamada Y (2018) In vivo reprogramming drives Kras-induced cancer development. Nat Commun 9:2081

Shore RE (1990) Overview of radiation-induced skin cancer in humans. Int J Radiat Biol 57:809–827

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821–5828

Sommer CA, Capilla A, Molina-Estevez FJ, Gianotti-Sommer A, Skvir N, Caballero I, Chowdhury S, Mostoslavsky G (2018) Modeling APC mutagenesis and familial adenomatous polyposis using human iPS cells. PLoS One 13:e0200657

Soto AM, Sonnenschein C (2011) The tissue organization field theory of cancer: a testable replacement for the somatic mutation theory. BioEssays 33:332–340

Soto AM, Sonnenschein C (2014) One hundred years of somatic mutation theory of carcinogenesis: is it time to switch? BioEssays 36:118–120

Szadvari I, Krizanova O, Babula P (2016) Athymic nude mice as an experimental model for cancer treatment. Physiol Res 65:S441–S453

Takahashi K, Yamanaka S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676

Wong DJ, Liu H, Ridky TW, Cassarino D, Segal E, Chang HY (2008) Module map of stem cell genes guides creation of epithelial cancer stem cells. Cell Stem Cell 2:333–344

Xu W, Zhao ZY, An QM, Dong B, Lv A, Li CP, Guan XY, Tian XY, Wu JH, Hao CY (2020) Comprehensive comparison of patient-derived xenograft models in hepatocellular carcinoma and metastatic liver Cancer. Int J Med Sci 17:3073–3081

Yamagiwa K, Ichikawa K (1918) Experimental study of the pathogenesis of carcinoma. J Cancer Res 3:1–29

Zhao W, Zhu Q, Tan P, Ajibade A, Long T, Long W, Li Q, Liu P, Ning B, Wang HY, Wang RF (2018) Tgfbr2 inactivation facilitates cellular plasticity and development of Pten-null prostate cancer. J Mol Cell Biol 10:316–330