International Journal of Biochemistry and Cell Biology 107 (2019) 62–68

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Induced pluripotent stem cells: A new strategy to model human cancer

S. Bindhya[a], C. Sidhanth[a], A. Shabna[a], S. Krishnapriya[a], M. Garg[b], T.S. Ganesan[a,*]

[a] Laboratory for Cancer Biology, Department of Medical Oncology and Clinical Research, Cancer Institute (WIA), Chennai, India
[b] Amity Institute of Molecular Medicine and Stem Cell Research (AIMMSCR), Amity University, Uttar Pradesh, India

ARTICLE INFO

Keywords:
iPSCs
Stem cells
Pathogenesis
Cancer
*In vitro*models

ABSTRACT

Induced pluripotent stem cells are derived from adult somatic cells by ectopic expression of stem cell factors OCT4, SOX2, MYC and KLF4. These cells have characteristic features similar to embryonic stem cells. Although there exists *in vitro* and *in vivo* models of cancer, recapitulating the earliest events in the pathogenesis remain challenging. More recently, induced pluripotent stem cells have been generated to model human disease and cancer. There are advantages in the cancer models derived from these cells as compared to existing conventional approaches. Induced pluripotent stem cells have been generated from cancer cell lines, primary tumours and from those with an inherited predisposition to develop cancer. In addition, these cells provide a valuable tool in understanding the pathogenesis of familial cancer in its earliest stages, and to identify additional genetic alterations that are required to develop cancer. Furthermore, these cells can serve as a resource in drug screening and developing new therapies.

1. Introduction

Models have always been used to understand the development and pathogenesis of cancer. In addition, they have been particularly useful in evaluating the efficacy of drugs used in the management of cancer. These include *in vitro* models such as cell lines, spheroids and organoids. Murine models have been the main basis for experimental cancer research for a long time. This review traces briefly the current model systems used to study malignant transformation. It also focuses on how the development of induced pluripotent stem cells can be an additional approach in understanding the pathogenesis of cancer. It can also serve as a defined *in vitro* model in drug development.

1.1. In vitro and in vivo models of carcinogenesis

Human cancer is often defined by a large number of genetic aberrations. Therefore, it is important to understand the genetic basis of the transformation of normal diploid cells to malignant. The most commonly used *in vitro* models were cancer cell lines generated from human tumours. Cell line-based models were helpful in the preclinical evaluation of the efficacy of cytotoxic drugs as well as in the understanding of the genetic changes (Barretina et al., 2012; Shoemaker, 2006). Additionally, isogenic cancer cell lines with knockout/knock-in of the specific gene provided an excellent tool for drug discovery and pharmacogenomics (Torrance et al., 2001; Di Nicolantonio et al., 2010). Moreover, a cell line derived xenograft models provided insights into pathogenesis, metastasis and angiogenesis of cancer, which aid in the development of therapies for the treatment of disease (Sausville and Burger, 2006). However, the major drawbacks of these models include the lack of tumour heterogeneity and acquired genetic aberrations due to long-term culture. Further, it has been observed that several cytotoxic drugs are active in xenograft murine models but have failed in clinical trials (Daniel et al., 2009).

Patient-derived xenograft (PDX) models are better in representing the genomic alterations, tumour heterogeneity, tumour microenvironment and angiogenesis compared to cell lines and xenografts (Reyal et al., 2012). Moreover, PDX models are good for testing the efficacy of cytotoxic drugs (McMillin et al., 2013) and in understanding the mechanisms responsible for drug resistance (Hidalgo et al., 2014). The major limitations of the PDX model are successful engraftment, variable growth rates, technical challenges and suitability for understanding early genetic events (Krumbach et al., 2011). Transgenic mice are valuable models for studying the biology of human diseases including cancers due to their phylogenetic relatedness to humans (Lampreht

Abbreviations: iPSCs, Induced Pluripotent Stem Cells; OCT4, Octamer -Binding Transcription Factor 4; SOX2, Sex Determining Region Y; KLF4, Krupple -Like Factor 4; SSEA, Stage-Specific Embryonic Antigen; TRA-1, Tumour Rejection Antigen 1; MEF, Mouse Embryonic Fibroblast; hTERT, Human Telomerase Reverse Transcriptase; NOD, Non-Obese Diabetic; SCID, Severe Combined Immunodeficiency; SV40, Simian Virus 40; CSC, Cancer Stem Cells

* Corresponding author at: Laboratory of Cancer Biology, Department of Medical Oncology and Clinical Research, Cancer Institute (WIA), 38 Sardar Patel Road, Guindy, 600036, Chennai, India.
E-mail addresses: tsganesan@gmail.com, ts.ganesan@cancerinstitutewia.org (T.S. Ganesan).

https://doi.org/10.1016/j.biocel.2018.12.008
Received 17 October 2018; Received in revised form 12 December 2018; Accepted 13 December 2018
Available online 14 December 2018
1357-2725/

Tratar et al., 2018), ease of maintaining and breeding them in the laboratory, and the availability of many inbred strains (Perlman, 2016). Some of the limitations of these models are the absence of metastasis, the time limitation for the accumulation of additional genetic lesions, high rate of mortality, challenges in expressing the exact mutations found in human tumours (Chen et al., 2005).

More recently, spheroids and organoids have gained prominence as another approach in modelling cancer. This approach is attractive because it is closer to what happens in human tumours. Spheroids are either grown on a three-dimensional (3D) biomatrix or in the suspension growth medium. Tumour cells grown in the 3D conditions imitate the avascular tumour nodules and recapitulate the tumour microenvironment which helps in the understanding of cell to cell interaction and angiogenesis (Hirschhaeuser et al., 2010). Spheroids develop hypoxic cores which results in necrosis, that mimics the poorly vascularised tumours *in vivo*. Hypoxia also stimulates complex signalling in cancer cells like HIF, PI3K, MAPK, and NFκB pathways and also lead to the formation of new tumour blood vessels (Weiswald et al., 2015). Similar to spheroid culture, tumour organoids are a 3D culture of cancer cells from the primary tumour grown in an appropriate matrix. Tumour organoids grow slowly and display heterogeneity similar to the original tumour and thus represent a good model for the assessment of drugs (Zanoni et al., 2016; Gao et al., 2015). Though the spheroids and organoid 3D culture serve as a good tumour model to understand the characteristics features of a tumour, it does not address the process of malignant transformation from normal cells.

1.2. Genetic approaches to model cancer from normal diploid cells

Pathologically, tumours originate from normal cells that accumulate genetic and epigenetic changes (Marusyk et al., 2012). The type and number of changes necessary for malignant transformation differ among tumour types. To understand the mechanism of transformation of normal cells into tumour cells, mouse cells were transformed into tumour cells by introducing specific genetic alterations (TP53, KRAS, Rb1) (Jacks et al., 1992). However, human cells were resistant to transformation with the same set of genes (Sager et al., 1983). Later, it was observed that human somatic cells have short telomeres and lack detectable telomerase activity in comparison to mouse embryonic cells (Zhao et al., 2004; Hahn et al., 1999; Hahn and Weinberg, 2002). The expression of telomerase in mouse embryonic fibroblast (MEF) cells allows them to divide and proliferate indefinitely. Therefore, minimal genetic alterations were sufficient to transform mouse cells rapidly (Kipling and Cooke, 1990; Allsopp et al., 1992). Similar attempts were made to transform normal human diploid cells. Normal human primary cells were immortalized initially with hTERT. Further, these immortalized cells in combination with oncogenes and tumour suppressor genes were able to accrue all changes necessary to invade and metastasize (Rangarajan and Weinberg, 2003; Boehm and Hahn, 2004; Kavsan et al., 2011). However, certain human cell types were resistant to such transformation for unknown reasons. Several studies also reported that the successful transformation of different human primary cells was possible without immortalization by hTERT (Seger et al., 2002). These results demonstrated that immortalization alone cannot fully account for the difference in the transformability of cell types. This clearly suggests that primary human cells have an undefined intrinsic mechanism, rendering them resistant to oncogenic transformation. Particularly, in the case of hereditary cancers, patients are predisposed to tumour development in specific tissues despite the fact that the mutant allele is present in all somatic cells. This suggests that the effects of cancer-relevant mutations are highly influenced by cell type-specific contexts in different environments. However, the major limitations of these models are their poor ability to propagate cell type and incapability of recapitulating the developmental process of cell transformation that occur *in vivo*. To study the process of transformation of a normal cell, one has to have the unlimited source of cells which represents the particular tissue type. This is now possible using induced pluripotent stem cells (iPSCs).

1.3. Cancer stem cells

There is now compelling evidence that cancer arises from a subpopulation of slow cycling, self-renewing, highly tumorigenic and therapy-resistant cells that have the ability to reconstitute a tumour in its entirety and are called as cancer stem cells (CSCs). The first convincing evidence for the presence of CSCs came from patients with acute myeloid leukaemia which could initiate hematopoietic malignancies in obese diabetic/severe combined immunodeficient mice (Bonnet and Dick, 1997). Since then CSCs have been identified in a variety of human malignancies including breast, ovary, brain, lung, colon and melanoma (Rycaj and Tang, 2015; Nagare et al., 2017). The proportion of CSCs are variable in different tumour types. For example, the proportion of CSCs are low in colon cancer whereas more were observed in melanoma. (Meacham and Morrison, 2013). To consider CSCs as one of the therapeutic target candidates for cancer, further studies are needed to address complexities and challenges involving their biological functions, biomarkers, signalling pathways and differentiation. Due to the scarcity, difficulty in isolation and maintaining them in long-term *in vitro* culture, they are poorly understood.

1.4. Induced pluripotent stem cells

Reprogramming a cancer cell into iPSCs enables to reset the identity of original cell types without altering the genome sequence of a cell. Therefore, it is possible to capture stem cell state from any cell line or tissue. Further, re-differentiation of cells to other tissue types would demonstrate the concept of cell type-specific carcinogenesis. Cellular reprogramming also helps us in studying the interactions between genetic and epigenetic drivers of oncogenesis. It has been shown that when normal cells are converted to iPSCs, there are substantial differences in expression of genes between the two. This difference has been explored by both micro array and RNA sequencing (Apostolou and Hochedlinger, 2013). However, the iPSCs retain some element of memory, as with appropriate growth factors, reconversion to the original lineage is easier (Kim et al., 2010). However, this has not been fully established with respect to transformation of either cancer cell lines or tumours to iPSCs. Typically, comparison of expression of genes between the two states alone has been performed (Chao and Chern, 2018). In this regard there is a difference between iPSCs derived from normal cells as opposed to cancer cells. Generation of iPSC from human cells represents an opportunity to develop *in vitro* models of carcinogenesis.

Induced pluripotent stem cells can be directly generated from post mitotic adult somatic cells. This is achieved by the forced expression of four stem cell specific transcription factors (OCT4, SOX2, KLF4, MYC) that reprograms the original differentiated cell into a stem cell (Takahashi and Yamanaka, 2006). In the field of regenerative medicine and cancer research, iPSCs hold great promise because a single cell can replace damaged or diseased cells. They have the ability to proliferate indefinitely and the potential to differentiate into any cell type in the body. Reprogramming of human somatic cells into iPSC solves several technical and ethical restrictions of using embryonic stem cells.

Technically, in the generation of iPSC few challenges remain such as efficiency, genome integration, incomplete reprogramming and tumour formation. However, considerable progress has been made to obtain iPSCs by improving the efficiency and safety. Reprogramming of somatic cells was initially achieved in most studies using viral expression vectors that mediate integration making reprogramming efficient in a short period of time (Okita et al., 2008; Hanna et al., 2008; Eminli et al., 2009). The conventional factors used for reprogramming such as CMYC and KLF4 have direct tumorigenic potential when overexpressed in a cell. To minimize this issue several other factors have been employed

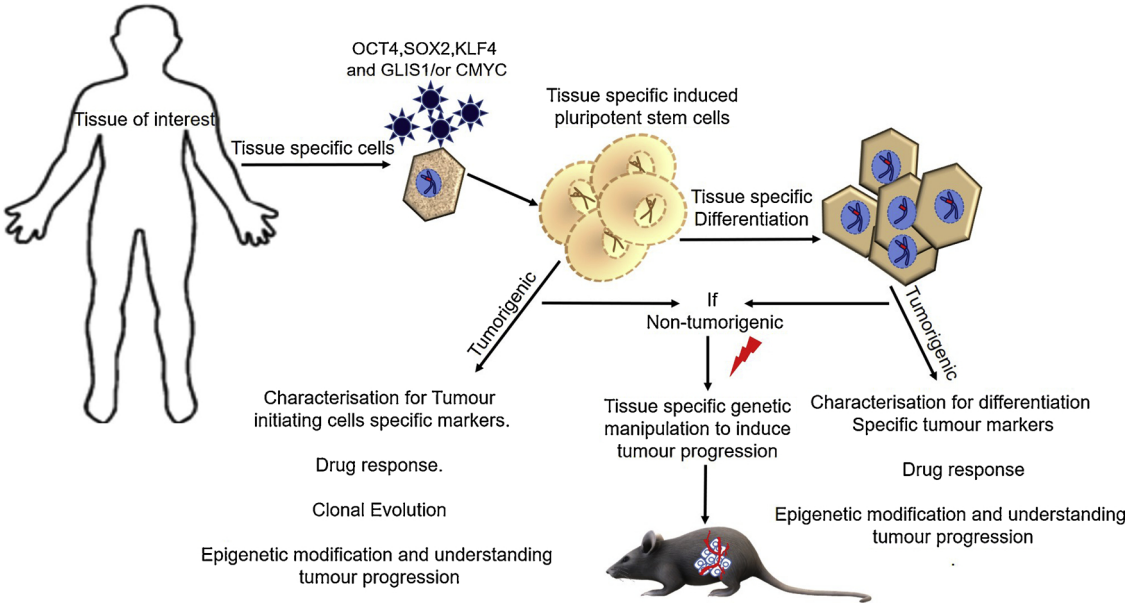

Fig. 1. Model to study carcinogenesis: Tissue specific cells isolated from patients predisposed to cancer can be reprogrammed to iPSC. Tumorigenic potential of both iPSC and tissue specific differentiated cells can be assessed. If these cells are not tumorigenic, then specific genetic changes could be introduced in these cells to induce tumour progression. This permits the development of both *in vitro* and *in vivo* cancer models.

for reprogramming. It has been reported that LIN28 in combination with OCT4 and SOX2 had led to the successful generation of iPSCs (Jung et al., 2014, Rais et al., 2013). Similarly, GLIS1 can replace CMYC in the conventional four factors (Maekawa and Yamanaka, 2011). However, random integration in the cell genome is the major limitation of the viral vector-based approach. Technical advances have led to the development of several techniques to reprogram cells minimizing DNA alterations (Seifinejad et al., 2010). Therefore, iPSC technology affords opportunities to study and understand stem cell properties and the embryonic development process (Boland et al., 2009).

Several iPSC models of diseases like neurodegenerative, haematological and familial syndromes, have been generated and disease-related information had been obtained using these cells (Ebert et al., 2012). If cancer stem cells or tumour initiating cells are the main driving force in a tumour, then by using iPSCs derived from the tissue of origin, it is possible to ascertain what genetic events are required to transform them (Fig. 1). Alternatively, it is possible to convert a malignant cell to an iPSC to study the differences between the two. Initially, efforts were made to reprogram malignant cell lines rather than primary tumours. The different reports led to some interesting conclusions. Mostly, there were an attempt to evaluate if the iPSCs had the ability to transform, the possibility of differentiation into corresponding lineages and sensitivity to cytotoxic drugs. In some reports, differences in the global expression of genes were also assessed.

1.5. Reprogramming of cancer cell lines

KBM7 (chronic myeloid leukaemia) cell line containing the BCR-ABL fusion oncogene was reprogrammed into iPSCs using the classical four transcription factors. Notably, MYC was essential and its absence did not result in reprogramming of cells. These results suggest that four factors are essential for reprogramming in certain cell types. KBM7-iPSCs were able to differentiate into hematopoietic lineages by expressing CD43 (T-cell marker), CD45 (hematopoietic lineage marker) and CD34 (stem cell marker). Interestingly, upon imatinib treatment, the KBM7-iPSCs lost dependency on BCR-ABL and acquired resistance to imatinib. These results indicate that imatinib targets cells that are more differentiated, which explains its inability to fully eradicate the disease in patients with chronic myeloid leukaemia (Carette et al., 2010).

Other cell lines that were converted into iPSCs included DLD-1 and HCT-116 (colorectal carcinoma), MIAPaCa-2 (pancreatic carcinoma) and hepatocellular carcinoma (PLC-3) using a retroviral approach with classical transcription factors (Miyoshi et al., 2010). These reprogrammed cells were called as post induced pluripotent cancer cells (Post-iPC). The DLD-1-Post-iPC cells expressed several differentiation markers like FABP4, MAP2 and PAX6. DLD1-Post-iPC cells were significantly more sensitive as compared to parental cells upon treatment with 5-fluorouracil (5- FU). However, the tumorigenic potential of DLD-1-Post-iPC cells in NOD/SCID mice was decreased as compared to parental cancer cells.

MCF-7 breast carcinoma cells were reprogrammed using four factors and were called as MCF-7/Rep cells. These cells exhibited a state between cancer cells and iPSCs after reprogramming. Expression of alkaline phosphatase was observed only in some MCF-7/Rep cells. These cells showed high expression of SOX2 and SSEA4, but moderate to low-level expression of OCT4, NANOG, SSEA3, TRA-1-81, and TRA-1-60. Moreover, MCF-7/Rep cells were unable to differentiate into three germ layers. The MCF-7/Rep cells which expressed elevated levels of SOX2 resembled CSC like cells and showed increased expression of CD44 and ALDH as compared to parental cells. Moreover, the SOX2 overexpressed cells had differential expression of the genes involved in mTOR signalling (Corominas-Faja et al., 2013). This study demonstrated that the overexpression of stem cell factors dysregulates mTOR signalling genes in partially reprogrammed cells enabling them to acquire cancer stem cell features.

Several sarcoma cell lines were reprogrammed (SAOS2, HOS,

Table 1
iPSCs models from Cancer Cell lines.

CANCER TYPE	CELL LINES	METHOD	FACTORS	RESULTS	TUMOUR FORMATION	REFERENCE
Chronic myeloid leukaemia	KBM7	Retrovirus	OCT4,SOX2, KLF4, MYC	1.Insensitivity to imatinib. 2.Express leukemic specific stem cells markers.	Not done.	(Carette et al., 2010)
Colorectal carcinoma	DLD1, HCT116	Retrovirus	OCT4,SOX2, KLF4, MYC	1.iPSCs from cancer cell lines expressed ES specific markers. 2.Sensitivity to cytotoxic drugs. 3.DLD-1-Post-iPC had Higher expression of p16 and p53 compared to the parental cells.	DLD-1-Post-iPC showed reduced invasion and tumorigenicity.	(Miyoshi et al., 2010)
Pancreatic carcinoma	MIApaCa2					
Hepatocellular carcinoma	PLC3					
Breast carcinoma	MCF-7	Retrovirus	OCT4,SOX2, KLF4, MYC	1.iPSCs exhibited an intermediate state and overexpressed SOX2. 2.Expressed high ALDH activity and CD44.	Not done	(Corominas-Faja et al., 2013)
Osteosarcoma	SAOS2, HOS, MG63	Lentivirus	OCT4,SOX2, KLF4,MYC, NANOG, LIN28	1.All sarcoma iPSCs expressed pluripotent markers. 2.Terminally differentiated into mature connective tissue and red blood cells. 3.Epigenetic modification observed in oncogenes and tumour suppressor gene.	Reduced Tumorigenicity.	(Zhang et al., 2013)
Ewing sarcoma	SK-NEP					
Liposarcoma	SW872					
Non-small cell lung cancer	H358, H460, IMR90	Lentivirus	OCT4,SOX2, KLF4, MYC	1.Reversed methylation. Dysregulated transcriptional activity.	Reduced tumorigenicity.	(Mahalingam et al.,2012)
Bladder	T24 HTB9	Sendai virus	OCT4,SOX2, KLF4, MYC	1.T24-iPSC express ES specific markers. 2.T24-iPSC differentiated into three lineages. 3.HTB-9 cells partially reprogrammed, instability in maintaining iPSC morphology.	Not done.	(Iskender et al., 2016)

MG63, SW782 and SKNEP) into iPSCs using pooled lentiviral particles for OCT4, SOX2, KLF4, NANOG, C-MYC and LIN28. iPSCs were able to differentiate into several mature connective tissues and hematopoietic cells. These differentiated cell types lost the tumorigenic potential, suggesting that epigenetic marks play a crucial role in tumorigenesis (Zhang et al., 2013). Several Non- small cell lung cancer cell line (H358, H460, IMR90), with abnormal karyotype were successfully reprogrammed into iPSCs. These studies demonstrated that reprogramming retunes the epigenetic marks in the cell lines and alters their tumorigenic ability (Mahalingam et al., 2012). Two different bladder carcinoma cell lines (T24 and HTB-9) were reprogrammed to iPSCs. The T24 cell line expressed all embryonic stem cell specific markers and was able to differentiate, while HTB-9 cells were partially reprogrammed with low expression of ES markers and lacked colony forming ability. This study demonstrates that, even in cell lines developed from the same organ sharing similar transcriptional network, differences in the epigenetic memory of the cell type decides reprogramming ability (Iskender et al., 2016) (Table 1).

1.6. Reprogramming primary malignant cells

Mononuclear cells were obtained from normal healthy donors and patients with chronic myeloid leukemia (CML). These mononuclear cells were reprogrammed into iPSCs through nucleofection of non-integrating episomal vectors. Blood cells were 100-fold more efficient in reprogramming than fibroblasts. CML-iPSCs showed a unique chromosomal translocation with embryonic stem cell phenotype and differentiation potential. This study shows that both the normal and CML bone marrow samples can be reprogrammed using episomal vectors with better efficiency in a short period of time while it is challenging with the virus-based approach (Hu et al., 2011).

In another report glioblastoma derived neural stem cells (GNS) were reprogrammed only with two factors OCT4 and KLF4 using the piggyBac transposon vector. The other two factors were already expressed in GNS. GNS derived iPSC showed extensive resetting of cancer-specific methylation. iPSCs upon differentiation into neural lineage were highly tumorigenic when injected into immunocompromised mice, whereas non-neural lineage did not display any malignant feature (Stricker et al., 2013). The above studies clearly proved that some reprogramming factors can be dispensed based on the cell type that endogenously expressed one of the four stem cell factors.

Furthermore, iPSCs have been generated from human skin fibroblasts of juvenile myelomonocytic leukemia (JMML) patients having a heterozygous p.E76 K missense mutation in PTPN11. When iPSCs were differentiated into different lineages of hematopoietic cell the mutation in PTPN11 was retained in the latter. Myeloid cells derived from iPSCs showed activation of STAT5 signalling along with the upregulation of miR-223, miR-15a (Gandre-Babbe et al., 2013; Mulero-Navarro et al., 2015). This study showed that iPSC can recapitulate pathological features of a disease and offers an unlimited resource for the evaluation of cytotoxic drugs.

There are no good models of pancreatic ductal adenocarcinoma (PDAC), to study the early events in its development. PDAC tissue specimens were reprogrammed into PDAC-iPSCs. PDAC-iPSCs were injected in immunodeficient mice which resulted in pancreatic intraepithelial neoplasia (early stage) to invasive stages. These teratomas secreted several proteins which include TGF-β, integrins and HNF4α from early to intermediate stages of PDAC. These results showed that iPSCs can be generated from organ-specific cells that could recapitulate the pre-malignant lesions of carcinogenesis (Kim et al., 2013). This is a good example of using iPSC cells to identify the biomarkers and pathways involved in the early progression of the disease. This approach can also be applied to other human malignancies (Table 2)..

Table 2
iPSCs models from primary tumour cells.

CANCER TYPE	PRIMARY CELLS	METHOD	FACTOR	RESULTS	TUMOUR FORMATION	REFERENCE
Chronic myeloid leukaemia	Mononuclear cells from bone marrow	Episomal	OCT4 SOX2 KLF4 MYC NANOG LIN28 SV40LT	CML-iPSCs showed ES specific features and maintained complex karyotype.	Not Done	(Hu et al., 2011)
Glioblastoma	Glioma neural stem cells	PiggyBac	OCT4 KLF4	Differentiated neural cells reset epigenetics of cells.	Differentiated neural lineage were malignant upon xenotransplantation.	(Stricker et al., 2013)
Juvenile myelomonocytic leukaemia	JMML-MNC- E76K missense in PTPN11 gene	Lentivirus	OCT4 SOX2 KLF4 MYC	1.Activation of STAT5 and its downstream signaling. 2.Upregulation of miR223 and miR15 A.	Not done	(Mulero- Navarro et al., 2015)
Pancreatic ductal adenocarcinoma	Pancreatic tissue	Lentivirus	OCT4 SOX2 KLF4 MYC	1.The teratomas from these iPSCs showed the early and invasive stages of PDAC.	Highly aggressive tumours *in vivo* which are mostly endodermal.	(Kim et al., 2013)

1.7. Reprogramming of somatic cells with germline mutations

Though these above models experimentally demonstrated the contribution of epigenetics in maintaining the malignant status, it did not help in understanding the process of malignant transformation of normal cells. To understand what additional genetic changes are required to transform a normal cell, one can take advantage of somatic cells with a germ line mutation. Familial predisposition to cancer is observed with the breast-ovary cancer syndrome (BRCA1 and 2), familial adenomatous polyposis (FAP) as two common examples. It is currently unclear the process of malignant transformation with any of the familial cancer predisposition syndromes. Generating iPSC from somatic cells from such patients will allow some of these questions to be addressed (Gingold et al., 2016).

For example, in BRCA1 associated cancer, skin fibroblasts were obtained from a large family (8 individuals) having BRCA1 mutations (5382insC) which were successfully reprogrammed to iPSCs by expressing OCT4, SOX2, KLF4, LIN28 and MYC using a non-integrating mRNA-based approach. All the iPSC lines showed expression of pluripotent markers such as TRA-1-60, TRA-1-81, SSEA3, and NANOG. These iPSCs were differentiated into ectoderm (positive for PAX6), mesoderm positive for (BRACHYURY and GATA4) and endoderm positive for (FOXA2, SOX17). The BRCA1-iPSC clones from these patients had an increased expression level of protein kinase C theta (PKC theta) (Soyombo et al., 2013).. These results were consistent with the previous study on breast cancer where they had found a high expression of PKC theta in ER-negative human BRCA1 mutant breast cancer cell line. Interestingly, there was no notable change in the morphology and growth between BRCA1 mutant iPSCs and BRCA wild-type. Further investigation to determine the cellular phenotype, genomic stability and tumorigenicity of BRCA1-iPSCs compared to BRCA1 parental fibroblasts were not performed.

Li-Fraumeni syndrome (LFS) is a familiar form of cancer caused by germline mutation in p53 (Malkin, 2011). The iPSCs were generated using fibroblasts obtained from a patient with LFS having a TP53 germline mutation (G245D) to understand its role in the pathogenesis of osteosarcoma (OS). The appropriate differentiation of LFS-iPSC preserved the hallmark features of OS such as impaired osteoblastic differentiation and tumour-forming ability. The LFS osteoblasts and LFS-iPSC derived osteoblasts showed decreased expression of the imprinted gene H19 during osteogenesis. Restoration of H19 expression in LFS-MSCs (mesenchymal stem cells) improved osteoblastic differentiation and suppressed the tumorigenicity (Lee et al., 2015). This study also identified gene signatures associated with clinical outcome.

Recently, APC mutant fibroblast cells from patients with familial adenomatous polyposis (FAP) [C1621 T and delAG4611 positions of the APC gene], had been reprogrammed to iPSCs using the traditional four factors (OCT4, SOX2, KLF4 and CMYC) (Sommer et al., 2018). It was observed that heterozygosity in the mutation of APC gene did not hinder the reprogramming of fibroblasts. It was also observed that the mutation of APC had not affected the proliferation rate and differentiation to intestinal cells in comparison to normal cells. Moreover, the expression of APC target genes was variable between iPSC derived cell lines from different donors and this variability was also observed within healthy donors. Further to minimize the inter individual genetic variability, isogenic APC iPSC lines were developed from normal cells using TALENS-mediated gene editing. These isogenic APC mutant cells upon differentiation to intestinal cells showed a major change in transcriptional activity along with dysregulation in key signalling pathways, which included abnormal lipid metabolism and increased cadherin expression. This study demonstrated the impact of mutant APC on the phenotypic and molecular characteristic of iPSC and their differentiated intestinal progeny. However, this study did not evaluate the impact of APC associated genes and their role in triggering colon cancer. Reprogramming can be applied to other familial cancers such as Lynch syndrome/hereditary non-polyposis colorectal cancer (HNPCC). Germline mutations of DNA mismatch repair genes such as MLH1, MSH2, MSH6, and PMS2, are frequent in HNPCC as well as in sporadic cancers (Bonadona et al., 2011; Gulati et al., 2011). Hence, reprogramming of non-cancerous somatic cells with HNPCC-germline mutations would be an alternative approach to study not only the pathogenesis of colon cancer but also the involvement of these genes in other cancers.

In these reports on iPSCs generated from patients with familial cancers several questions remain. First, the cells utilized for reprogramming were fibroblasts rather than cells from which a tumour arises. For example, in the case of BRCA associated inherited cancers, primary breast or ovarian epithelial cells would have been a better choice, rather than fibroblast. Similarly, instead of fibroblasts from patients with FAP, it could have been primary colon epithelial cells. Further, one can develop iPSCs from different tissues to understand why tumours develop predominantly in breast or ovary (BRCA) or colon (FAP). Finally, as these iPSCs are generally unable to form malignant tumours, it is possible to address what additional genetic changes are required to transform them in a defined manner. This will be similar to experiments done previously to transform normal diploid cells, the only difference being that stem cells are being used in the assumption that they may be the initial target. Therefore, iPSCs may be helpful in being

Table 3
iPSCs models from patients predisposed to cancer.

CANCER TYPE	PRIMARY CELLS	METHOD	FACTOR	RESULTS	TUMOUR FORMATION	REFERENCE
Breast ovarian cancer syndrome BRCA1 mutant Cells	5382insC skin Fibroblast	miRNA	OCT4 SOX2 KLF4 LIN28 MYC	1.iPSC cells showed increased expression of PKC theta. 2.No difference in morphology as compared to wild type.	Not done	(Soyombo et al., 2013)
Li-Fraumeni syndrome (LFS) p53 mutant cells	G245D missense in p53 gene – Skin Fibroblast	Sendai virus	OCT4 SOX2 KLF4 MYC	1.LFS-iPSCs recapitulated features of osteosarcoma. 2.H19 expression was upregulated in WT- p53 osteoblast compared to LFS-Osteoblast. 3.LFS-iPSCs showed defective osteoblastic differentiation.	LFS-Osteoblast induced tumorigenesis compared to WT. Restoration of H19 in LFS-MSCs reduced tumour incidence.	(Lee et al., 2015)
Familial adenomatous polyposis Cancer (FAP)-APC mutant cells	c.1621C > T del – FAP Skin fibroblast c.4611delAG -– FAP skin fibroblast c.1246del146- isogenic APC mutant	Lentivirus	OCT4 SOX2 KLF4 MYC	1.FAP-iPSCs expressed all ES specific markers, and differentiated into three germ layer cells. 2.Isogenic APC mutant iPSC showed significant difference in transcriptional activity of many intestinal specific genes. 3.Isogenic APC mutant iPSC showed dysregulated signaling pathways.	Not done	(Sommer et al., 2018)

an additional model to study the pathogenesis of cancer in all aspects (Table 3).

2. Conclusion

Deriving iPSCs from either the primary tumour or an established cell line may be helpful in delineating the earliest steps of tumorigenesis. However, to evaluate epigenetic changes that maintain the transformed state iPSC models are excellent as all the epigenetic marks are reset during reprogramming. Alternatively, one can derive iPSC models from somatic cells with a germline mutation. These iPSC models allow one to study what additional changes are required to transform these cells. In addition, it is possible to address the mechanism behind tissue specificity of a particular inherited cancer. So far, we still do not understand the mechanistic basis of this selectivity.

Acknowledgements

We acknowledge Indian Council of Medical Research (ICMR) and Department of Biotechnology, Government of India for their financial support. We declare that there are no conflicts of interest associated with this work.

References

Allsopp, R.C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E.V., Futcher, A.B., Greider, C.W., Harley, C.B., 1992. Telomere length predicts replicative capacity of human fibroblasts. Proc. Natl. Acad. Sci. U. S. A. 89, 10114–10118.
Apostolou, E., Hochedlinger, K., 2013. Chromatin dynamics during cellular reprogramming. Nature 502, 462–471.
Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A.A., Kim, S., Wilson, C.J., Lehar, J., Kryukov, G.V., Sonkin, D., Reddy, A., Liu, M., Murray, L., Berger, M.F., Monahan, J.E., Morais, P., Meltzer, J., Korejwa, A., Jane-Valbuena, J., Mapa, F.A., Thibault, J., Bric-Furlong, E., Raman, P., Shipway, A., Engels, I.H., Cheng, J., Yu, G.K., Yu, J., Aspesi Jr, P., De Silva, M., Jagtap, K., Jones, M.D., Wang, L., Hatton, C., Palescandolo, E., Gupta, S., Mahan, S., Sougnez, C., Onofrio, R.C., Liefeld, T., Macconaill, L., Winckler, W., Reich, M., Li, N., Mesirov, J.P., Gabriel, S.B., Getz, G., Ardlie, K., Chan, V., Myer, V.E., Weber, B.L., Porter, J., Warmuth, M., Finan, P., Harris, J.L., Meyerson, M., Golub, T.R., Morrissey, M.P., Sellers, W.R., Schlegel, R., Garraway, L.A., 2012. The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 483, 603–607.
Boehm, J.S., Hahn, W.C., 2004. Immortalized cells as experimental models to study cancer. Cytotechnology 45, 47–59.
Boland, M.J., Hazen, J.L., Nazor, K.L., Rodriguez, A.R., Gifford, W., Martin, G., Kupriyanov, S., Baldwin, K.K., 2009. Adult mice generated from induced pluripotent stem cells. Nature 461, 91–94.
Bonadona, V., Bonaiti, B., Olschwang, S., Grandjouan, S., Huiart, L., Longy, M., Guimbaud, R., Buecher, B., Bignon, Y.J., Caron, O., Colas, C., Nogues, C., Lejeune-Dumoulin, S., Olivier-Faivre, L., Polycarpe-Osaer, F., Nguyen, T.D., Desseigne, F., Saurin, J.C., Berthet, P., Leroux, D., Duffour, J., Manouvrier, S., Frebourg, T., Sobol, H., Lasset, C., Bonaiti-Pellie, C., French Cancer Genetics, N., 2011. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA 305, 2304–2310.
Bonnet, D., Dick, J.E., 1997. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. 3, 730–737.
Carette, J.E., Pruszak, J., Varadarajan, M., Blomen, V.A., Gokhale, S., Camargo, F.D., Wernig, M., Jaenisch, R., Brummelkamp, T.R., 2010. Generation of iPSCs from cultured human malignant cells. Blood 115, 4039–4042.
Chao, H.M., 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, Z., Trotman, L.C., Shaffer, D., Lin, H.K., Dotan, Z.A., Niki, M., Koutcher, J.A., Scher, H.I., Ludwig, T., Gerald, W., Cordon-Cardo, C., Pandolfi, P.P., 2005. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 436, 725–730.
Corominas-Faja, B., Cufi, S., Oliveras-Ferraros, C., Cuyas, E., Lopez-Bonet, E., Lupu, R., Alarcon, T., Vellon, L., Iglesias, J.M., Leis, O., Martin, A.G., Vazquez-Martin, A., Menendez, J.A., 2013. Nuclear reprogramming of luminal-like breast cancer cells generates Sox2-overexpressing cancer stem-like cellular states harboring transcriptional activation of the mTOR pathway. Cell Cycle 12, 3109–3124.
Daniel, V.C., Marchionni, L., Hierman, J.S., Rhodes, J.T., Devereux, W.L., Rudin, C.M., Yung, R., Parmigiani, G., Dorsch, M., Peacock, C.D., Watkins, D.N., 2009. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. Cancer Res. 69, 3364–3373.
Di Nicolantonio, F., Arena, S., Gallicchio, M., Bardelli, A., 2010. Isogenic mutant human cells: a new tool for personalized cancer medicine. Cell Cycle 9, 20–21.
Ebert, A.D., Liang, P., Wu, J.C., 2012. Induced pluripotent stem cells as a disease modeling and drug screening platform. J. Cardiovasc. Pharmacol. 60, 408–416.
Eminli, S., Foudi, A., Stadtfeld, M., Maherali, N., Ahfeldt, T., Mostoslavsky, G., Hock, H., Hochedlinger, K., 2009. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. Nat. Genet. 41, 968–976.
Gandre-Babbe, S., Paluru, P., Aribeana, C., Chou, S.T., Bresolin, S., Lu, L., Sullivan, S.K., Tasian, S.K., Weng, J., Favre, H., Choi, J.K., French, D.L., Loh, M.L., Weiss, M.J., 2013. Patient-derived induced pluripotent stem cells recapitulate hematopoietic abnormalities of juvenile myelomonocytic leukemia. Blood 121, 4925–4929.
Gao, H., Korn, J.M., Ferretti, S., Monahan, J.E., Wang, Y., Singh, M., Zhang, C., Schnell, C., Yang, G., Zhang, Y., Balbin, O.A., Barbe, S., Cai, H., Casey, F., Chatterjee, S., Chiang, D.Y., Chuai, S., Cogan, S.M., Collins, S.D., Dammassa, E., Ebel, N., Embry, M., Green, J., Kauffmann, A., Kowal, C., Leary, R.J., Lehar, J., Liang, Y., Loo, A., Lorenzana, E., Robert Mcdonald 3rd, E., Mclaughlin, M.E., Merkin, J., Meyer, R., Naylor, T.L., Patawaran, M., Reddy, A., Roelli, C., Ruddy, D.A., Salangsang, F., Santacroce, F., Singh, A.P., Tang, Y., Tinetto, W., Tobler, S., Velazquez, R., Venkatesan, K., Von Arx, F., Wang, H.Q., Wang, Z., Wiesmann, M., Wyss, D., Xu, F., Bitter, H., Atadja, P., Lees, E., Hofmann, F., Li, E., Keen, N., Cozens, R., Jensen, M.R., Pryer, N.K., Williams, J.A., Sellers, W.R., 2015. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat. Med. 21, 1318–1325.
Gingold, J., Zhou, R., Lemischka, I.R., Lee, D.F., 2016. Modeling cancer with pluripotent stem cells. Trends Cancer 2, 485–494.
Gulati, S., Gustafson, S., Daw, H.A., 2011. Lynch syndrome associated with PMS2 mutation: understanding current concepts. Gastrointest Cancer Res. 4, 188–190.
Hahn, W.C., Weinberg, R.A., 2002. Modelling the molecular circuitry of cancer. Nat. Rev. Cancer 2, 331–341.
Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergen, R.L., Brooks, M.W., Weinberg,

R.A., 1999. Creation of human tumour cells with defined genetic elements. Nature 400, 464–468.
Hanna, J., Markoulaki, S., Schorderet, P., Carey, B.W., Beard, C., Wernig, M., Creyghton, M.P., Steine, E.J., Cassady, J.P., Foreman, R., Lengner, C.J., Dausman, J.A., Jaenisch, R., 2008. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. Cell 133, 250–264.
Hidalgo, M., Amant, F., Biankin, A.V., Budinska, E., Byrne, A.T., Caldas, C., Clarke, R.B., De Jong, S., Jonkers, J., Maelandsmo, G.M., Roman-Roman, S., Seoane, J., Trusolino, L., Villanueva, A., 2014. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov 4, 998–1013.
Hirschhaeuser, F., Menne, H., Dittfeld, C., West, J., Mueller-Klieser, W., Kunz-Schughart, L.A., 2010. Multicellular tumor spheroids: an underestimated tool is catching up again. J. Biotechnol. 148, 3–15.
Hu, K., Yu, J., Suknuntha, K., Tian, S., Montgomery, K., Choi, K.D., Stewart, R., Thomson, J.A., Slukvin, Ii, 2011. Efficient generation of transgene-free induced pluripotent stem cells from normal and neoplastic bone marrow and cord blood mononuclear cells. Blood 117, e109–19.
Iskender, B., Izgi, K., Canatan, H., 2016. Reprogramming bladder cancer cells for studying cancer initiation and progression. Tumour Biol. 37, 13237–13245.
Jacks, T., Fazeli, A., Schmitt, E.M., Bronson, R.T., Goodell, M.A., Weinberg, R.A., 1992. Effects of an Rb mutation in the mouse. Nature 359, 295–300.
Kavsan, V.M., Iershov, A.V., Balynska, O.V., 2011. Immortalized cells and one oncogene in malignant transformation: old insights on new explanation. BMC Cell Biol. 12, 23.
Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I., Yabuuchi, A., Takeuchi, A., Cunniff, K.C., Hongguang, H., Mckinney-Freeman, S., Naveiras, O., Yoon, T.J., Irizarry, R.A., Jung, N., Seita, J., Hanna, J., Murakami, P., Jaenisch, R., Weissleder, R., Orkin, S.H., Weissman, I.L., Feinberg, A.P., Daley, G.Q., 2010. Epigenetic memory in induced pluripotent stem cells. Nature 467, 285–290.
Kim, J., Hoffman, J.P., Alpaugh, R.K., Rhim, A.D., Reichert, M., Stanger, B.Z., Furth, E.E., Sepulveda, A.R., Yuan, C.X., Won, K.J., Donahue, G., Sands, J., Gumbs, A.A., Zaret, K.S., 2013. An iPSC line from human pancreatic ductal adenocarcinoma undergoes early to invasive stages of pancreatic cancer progression. Cell Rep. 3, 2088–2099.
Kipling, D., Cooke, H.J., 1990. Hypervariable ultra-long telomeres in mice. Nature 347, 400–402.
Krumbach, R., Schuler, J., Hofmann, M., Giesemann, T., Fiebig, H.H., Beckers, T., 2011. Primary resistance to cetuximab in a panel of patient-derived tumour xenograft models: activation of MET as one mechanism for drug resistance. Eur. J. Cancer 47, 1231–1243.
Lee, D.F., Su, J., Kim, H.S., Chang, B., Papatsenko, D., Zhao, R., Yuan, Y., Gingold, J., Xia, W., Darr, H., Mirzayans, R., Hung, M.C., Schaniel, C., Lemischka, I.R., 2015. Modeling familial cancer with induced pluripotent stem cells. Cell 161, 240–254.
Maekawa, M., Yamanaka, S., 2011. Glis1, a unique pro-reprogramming factor, may facilitate clinical applications of iPSC technology. Cell Cycle 10, 3613–3614.
Mahalingam, D., Kong, C.M., Lai, J., Tay, L.L., Yang, H., Wang, X., 2012. Reversal of aberrant cancer methylome and transcriptome upon direct reprogramming of lung cancer cells. Sci. Rep. 2, 592.
Malkin, D., 2011. Li-fraumeni syndrome. Genes Cancer 2, 475–484.
Marusyk, A., Almendro, V., Polyak, K., 2012. Intra-tumour heterogeneity: a looking glass for cancer? Nat. Rev. Cancer 12, 323–334.
Mcmillin, D.W., Negri, J.M., Mitsiades, C.S., 2013. The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. Nat. Rev. Drug Discov. 12, 217–228.
Meacham, C.E., Morrison, S.J., 2013. Tumour heterogeneity and cancer cell plasticity. Nature 501, 328–337.
Miyoshi, N., Ishii, H., Nagai, K., Hoshino, H., Mimori, K., Tanaka, F., Nagano, H., Sekimoto, M., Doki, Y., Mori, M., 2010. Defined factors induce reprogramming of gastrointestinal cancer cells. Proc. Natl. Acad. Sci. U. S. A. 107, 40–45.
Mulero-Navarro, S., Sevilla, A., Roman, A.C., Lee, D.F., D'souza, S.L., Pardo, S., Riess, I., Su, J., Cohen, N., Schaniel, C., Rodriguez, N.A., Baccarini, A., Brown, B.D., Cave, H., Caye, A., Strullu, M., Yalcin, S., Park, C.Y., Dhandapany, P.S., Yongchao, G., Edelmann, L., Bahieg, S., Raynal, P., Flex, E., Tartaglia, M., Moore, K.A., Lemischka, I.R., Gelb, B.D., 2015. Myeloid dysregulation in a human induced pluripotent stem cell model of PTPN11-associated juvenile myelomonocytic leukemia. Cell Rep. 13, 504–515.
Nagare, R.P., Sneha, S., Priya, S.K., Ganesan, T.S., 2017. Curr. Stem Cell Res. Ther. 12 (1), 37–44.
Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., Yamanaka, S., 2008. Generation of mouse induced pluripotent stem cells without viral vectors. Science 322, 949–953.
Perlman, R.L., 2016. Mouse models of human disease: an evolutionary perspective. Evol. Med. Publ. Health 2016, 170–176.
Rangarajan, A., Weinberg, R.A., 2003. Opinion: comparative biology of mouse versus human cells: modelling human cancer in mice. Nat. Rev. Cancer 3, 952–959.
Reyal, F., Guyader, C., Decraene, C., Lucchesi, C., Auger, N., Assayag, F., De Plater, L., Gentien, D., Poupon, M.F., Cottu, P., De Cremoux, P., Gestraud, P., Vincent-Salomon, A., Fontaine, J.J., Roman-Roman, S., Delattre, O., Decaudin, D., Marangoni, E., 2012. Molecular profiling of patient-derived breast cancer xenografts. Breast Cancer Res. 14, R11.
Rycaj, K., Tang, D.G., 2015. Cell-of-origin of cancer versus cancer stem cells: assays and interpretations. Cancer Res. 75, 4003–4011.
Sager, R., Tanaka, K., Lau, C.C., Ebina, Y., Anisowicz, A., 1983. Resistance of human cells to tumorigenesis induced by cloned transforming genes. Proc. Natl. Acad. Sci. U. S. A. 80, 7601–7605.
Sausville, E.A., Burger, A.M., 2006. Contributions of human tumor xenografts to anticancer drug development. Cancer Res. 66, 3351–3354 discussion 3354.
Seger, Y.R., Garcia-Cao, M., Piccinin, S., Cunsolo, C.L., Doglioni, C., Blasco, M.A., Hannon, G.J., Maestro, R., 2002. Transformation of normal human cells in the absence of telomerase activation. Cancer Cell 2, 401–413.
Seifinejad, A., Tabebordbar, M., Baharvand, H., Boyer, L.A., Salekdeh, G.H., 2010. Progress and promise towards safe induced pluripotent stem cells for therapy. Stem Cell Rev. 6, 297–306.
Shoemaker, R.H., 2006. The NCI60 human tumour cell line anticancer drug screen. Nat. Rev. Cancer 6, 813–823.
Sommer, C.A., Capilla, A., Molina-Estevez, F.J., 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.
Soyombo, A.A., Wu, Y., Kolski, L., Rios, J.J., Rakheja, D., Chen, A., Kehler, J., Hampel, H., Coughran, A., Ross, T.S., 2013. Analysis of induced pluripotent stem cells from a BRCA1 mutant family. Stem Cell Rep. 1, 336–349.
Stricker, S.H., Feber, A., Engstrom, P.G., Caren, H., Kurian, K.M., Takashima, Y., Watts, C., Way, M., Dirks, P., Bertone, P., Smith, A., Beck, S., Pollard, S.M., 2013. Widespread resetting of DNA methylation in glioblastoma-initiating cells suppresses malignant cellular behavior in a lineage-dependent manner. Genes Dev. 27, 654–669.
Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676.
Torrance, C.J., Agrawal, V., Vogelstein, B., Kinzler, K.W., 2001. Use of isogenic human cancer cells for high-throughput screening and drug discovery. Nat. Biotechnol. 19, 940–945.
Lampreht Tratar, U., Horvat, S., Cemazar, M., 2018. Transgenic mouse models in cancer research. Front. Oncol. 8, 268.
Weiswald, L.B., Bellet, D., Dangles-Marie, V., 2015. Spherical cancer models in tumor biology. Neoplasia 17, 1–15.
Zanoni, M., Piccinini, F., Arienti, C., Zamagni, A., Santi, S., Polico, R., Bevilacqua, A., Tesei, A., 2016. 3D tumor spheroid models for in vitro therapeutic screening: a systematic approach to enhance the biological relevance of data obtained. Sci. Rep. 6, 19103.
Zhang, X., Cruz, F.D., Terry, M., Remotti, F., Matushansky, I., 2013. Terminal differentiation and loss of tumorigenicity of human cancers via pluripotency-based reprogramming. Oncogene 32, 2249–2260 2260 e1-21.
Zhao, J.J., Roberts, T.M., Hahn, W.C., 2004. Functional genetics and experimental models of human cancer. Trends Mol. Med. 10, 344–350.