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PSC-Dependent Stem Cell Engineering for Management of Cancer and Its Current Challenges

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Abstract:
Stem cells are not only used for regenerative medicine, but also considered as a useful tool for cancer therapy. Pluripotent stem cells are promising tools in these kinds of therapies because of their stemness properties (that includes unlimited self-renewal & ability to differentiate into all cell types in the body). After discovery, the potentials of induced Pluripotent stem cells (iPSCs) have been explored by several researchers to unravel the molecular mechanism responsible for cancer initiation and progression.
A rare population of cells within tumor, known as Cancer stem cells (CSCs), exhibit stemness & phenotypic plasticity that are mainly responsible for resistance to chemotherapy, metastasis, radiotherapy, cancer development & tumor relapse. Off late, stem cells are being engineered to carry therapeutic reagents to target the tumor sites. Cancer vaccines based on knowledge of CSCs have been studied and applied for cancer treatment. Further applications of this technology include high-throughput drug screening, epigenetic reprogramming of cancer cells to normal, immunotherapy & regenerative cell therapies. iPSCs have been used to create active T cells to support cancer immunotherapy.
The similarities between CSCs and Pluripotent stem cells (PSCs) can provide an appropriate source of CSC specific antigens by cultivation of PSCs which could help in the development of prophylactic & therapeutic cancer vaccine. This review discusses induced pluripotent stem cells, stem cell applications in transplantation, cancer stem cells, comparison between PSCs to CSCs, potential of stem cells engineering to revolutionize cancer treatment and current challenges on clinical applications.

Keywords: Stem cells, Cancer, Embryonic stem cells, Cancer stem cells, Stem cell therapy, Cancer vaccine, Pluripotency

Background:
Reprogramming somatic cells has a long history. In the year of 1966, when adult frog was developed by the nuclear transplantation of differentiated somatic cell nuclei (John Gurdon). (i) After this, many animal models including generation of a sheep, named Dolly (Dr. Wilmut *et al.*,1996) from the cells which were derived from adult mammary glands. (ii) Discovery of human embryonic stem cells (hESCs)

in the year 1998 (Dr. James Thomson) was revolutionary in the field of stem cells & regenerative medicine. (iii) In 2006, Takashaki & Yamanaka reported the reprogramming of murinefibroblast by ectopic expression of Oct4, Sox2, Klf4 & c-Myc. (iv) In 2007, two independent research groups found that iPSCs exhibit similar morphological, proliferative, genomic & phenotypic characteristics as hESCs. (v) In 2018, Dr. Pablo Ross & his team achieved a significant breakthrough as they used 'in vivo reprogramming' to transform fetal sheep cells into more embryonic stage, leading to birth of viable lambs.

Introduction:

Cancer is a major health issue worldwide & leading cause of death. According to some scientific mathematical estimations of WHO (WHO report on cancer, 2020), worldwide cancer rate may exceed 29 million cases annually with approximately 1 out of 6 deaths in 2040. Various factors such as failure of current treatments, drug resistance (Vasan *et al*., 2019), delayed and/or wrong diagnosis (Brand, N.R. *et al*.,2019), absence of strong immune system in affected patients (Otto-Meyer, S., *et al*.,2019). Main causes of cancer deaths are metastasis post treatment and recurrence of tumors after resection.

Our bodies contain a pool of stem cells. Organs and tissues are made up of specialized cells from the pool of stem cells that form shortly after fertilization. Two main characteristics of stem cells are: ability to self-renew & to differentiate. There are several types of stem cells, for example, embryonic stem cells (ESCs) exist only at the earliest stage of embryo, but adult stem cells, which are retained throughout life, appear during the foetal development.

Recently, scientists have discovered how to form induced pluripotent stem cells. This is done by re-activating critical genes that define embryonic stem cells to make adult stem cells to revert to an embryonic like state of pluripotency.

Currently, the role of CSCs in propagation of malignant tumours & resistance to therapy has become more accentuated. Beside their ability both to self-renew and differentiate into many heterogeneous cells with lesser potency, major disorders related to malignancy such as drug resistance, plasticity, EMT (Epithelial to mesenchymal transition) & metastasis, and tumour recurrence are attributed to the existence of CSCs (Akbarzadeh, M., *et al*., 2019). Several attempts already have been made to isolate and distinguish Cancer Stem Cells to enable more advanced diagnosis and treatment, but, lack of specific markers for cellular recognition is a huge obstacle in dealing with CSCs.

PSCs, with an indefinite self-renewal capacity & capability to generate all cell types of the body, have several resemblances with CSCs. However, PSC-based cancer therapy studies are restricted to derivation of functional cancer fighting cells that include PSC-derived dendritic cells (Yanagimachi, M.D., *et al*., 2013).

Until now, there are many methods to treat cancer including chemotherapy, radiation, immunotherapy, surgical extraction, targeted drug therapy and stem cells. Stem cells have contributed notable roles for those approaches of cancer management. Stem cells have been exploited as useful tool in supporting conventional approaches as well as developing new methods for the cancer therapy.

Stem cell transplantation to recover the immune system:

Stem cell transplantation (SCT) can recover marrow function for the patients who have serious marrow injuries or damaged immune. Stem cell for the SCT procedure can come from the bone marrow, umbilical cord blood or peripheral blood.

In autologous SCT, patients use their own stem cells. High doses of radiation therapy or chemotherapy are used to eliminate the cancer cells, but these can severely damage the bone marrow & immune system of the patient. Autologous SCT is used here. Here the stem cells are collected from bone marrow or blood before treatment, then frozen. Later, thawed stem cells are reinfused into patient to restore the function of immune system (Illerhaus et al., 2006; Kessinger et al., 1991) (**Fig. 1**).
However, those could increase the risk of relapse. Recovered immune system could be stronger, but there would be no ability to eliminate remaining cancer cells (Igney and Krammer, 2002).

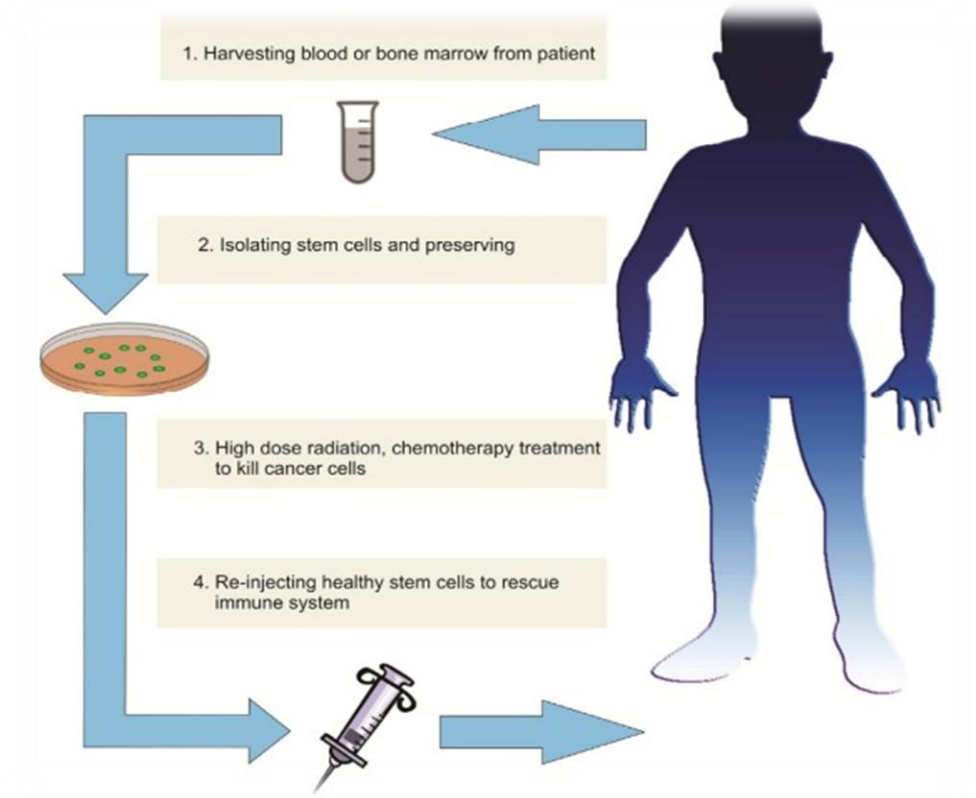

Figure 1. **Autologous stem cells transplantation in the cancer treatment**. (Nguyen *et al.,* 2015)

In allogenic SCT, stem cells from related or unrelated donors are used. A donor maybe a family member. Tissue typing uses HLA (Human Leukocyte Antigens). Through the HLA test, we can compare patient's blood & tissue type against a donor's blood samples. After destroying cancer cells by high dose of chemotherapy or radiation therapy, patient becomes ready for transplant. After entering bloodstream, stem cells move to bone marrow and start to produce new blood cells. This process is termed as engraftment. An allogenic SCT also carries the risk of Graft-versus-host-disease (GVHD), a condition where donated cells attack the tissues.

Origin of Cancer Stem Cells (CSCs):
Many evidences support the idea of representing the cancer as a stem cell malignancy & introduction of CSCs as being responsible for the metastasis & tumor recurrence. Proposed mechanisms for these malignancies: Cell-cell fusion, genetic instability, horizontal gene transfer & cell-cell microenvironment. There are many assumptions about the origin of Cancer stem cells which include disrupted tissue

resident stem cells such as specific adult stem cells & their precursors, improperly activated residual embryonic cells in adult tissues & dedifferentiated somatic cells. (Wu *et al*., 2008)

Comparison between Pluripotent Stem Cells (PSCs) and Cancer Stem Cells (CSCs):

The stemness characteristics and the similarities between CSCs and early developmental cells are remarkable in the stem cell theory of cancer (**Fig. 2**). In several ways, the cancer cells imitate early developmental pathways at the biological & molecular levels. The similarities in protein content and metabolism can show the proximity of CSCs and PSCs. Moreover, the signaling pathways & the Transcription factor (TF) expressions (Hadjimichael, C., *et al*., 2015) associated with pluripotency both in PSCs and CSCs are close enough.

Important Transcription Factors:

The induction of main pluripotency associated Transcription Factors Oct4, Sox2, KLF4 & NANOG results in induction of the pluripotency in terminally differentiated cells into iPSCs. These TFs are the key controllers of stemness in CSCs (Muller, M., *et al*., 2016).

Signaling pathways:

Two main characteristics of both CSCs and PSCs are: Self-renewal and proliferation. Both cells adjust the self-renewal process through same signaling pathways (Notch, WNT, Hedgehog). Irregular expressions of these key factors in CSCs disrupt balanced state & make them prone to tumor formation (Dressen *et al*., 2007).

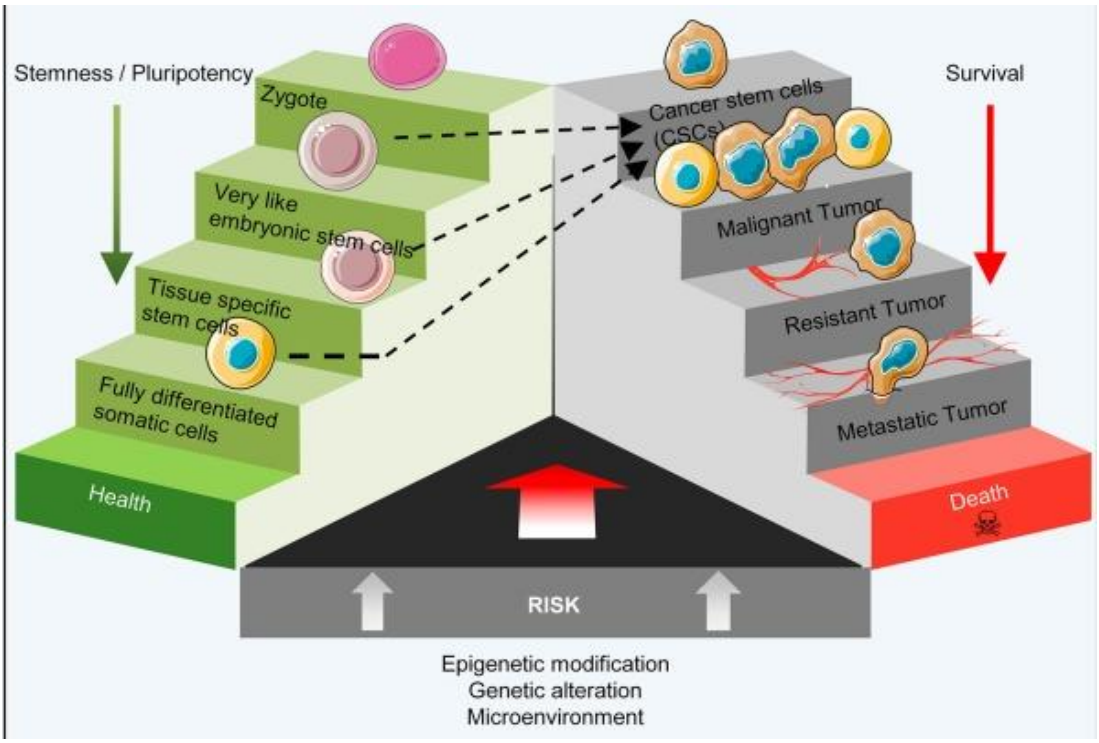

Figure 2. In the early embryo, to create a new being, the most important factors are stemness and pluripotency. In some cells (for example, very small embryonic-like stem cells or VSELs & tissue specific stem cells in adult tissues), activation of pluripotency leads to formation of CSCs. (Barati *et al.*,2021)

Mechanisms of iPSCs-mediated treatment of cancer:

We are living in the post-genomic era, but still there are many problems in the field of cancer & molecular cell biology that need to be solved. Still, we are not able to eradicate cancer completely. Most challenging one is the metastatic cancer as there are very few therapeutic options available & majority of the patients show severe side-effects resulting in mortality because of treatment itself. But iPSCs show the potential to cure cancer. (S.J. Sharkis *et al*., 2012)

There are mainly four possible scenarios where iPSCs could help as shown in **Fig. 3**: I) modeling cancer pathogenesis and drug screening, II) reprogramming the cancer cells back to normal phenotype, III) cancer immunotherapy, IV) regenerative cell therapy.

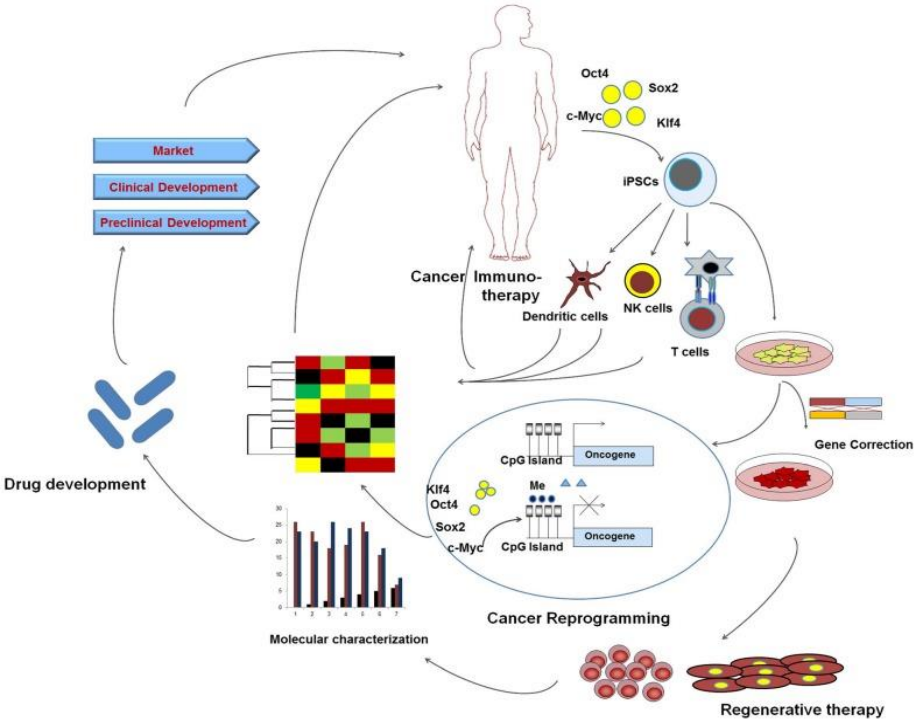

Figure 3. Applications of iPSCs in cancer therapy. Induced pluripotent stem cells derived from cancer cells can be differentiated into T cells, Dendritic cells, and Natural killer cells which can suppress or destroy tumor. Moreover, gene editing technology could also be used to correct cancer mutation in iPSCs. (Potdar and Chaudhary, Applied cancer research, 2017, 37:5)

Production of Cancer-specific T cells from iPSCs:

Molecular mechanism responsible for malignant transformations still not clearly understood. Cancer-specific mutations, deletions, translocations are generally associated to particular tissue type which indicate that the effect of these mutations are not only influenced by environmental factors but also dictated heavily by epigenetic states of a cell. So, iPSCs provide a platform to make out the association of oncogenic mutations with several tissue types & how these mutations dictate the malignant fate of the cell. (Baitsch *et al*., 2011; Severson *et al*., 2015).

T lymphocytes play very important role in immune system and are at the core of adaptive immunity to response to specific invaders. In case of cancer, T cells find out and eliminate targeted cancer cells. Several ways are there to generate cancer cells specific killer T cells through reprogramming techniques. Generating iPSCs from mature CD8+ T cells, is one of those methods (**Fig. 4a**).

Mature killer T cells (T lymphocytes) are reprogrammed into induced Pluripotent Stem Cells (iPSCs) by exposing them into specific factors, known as Yamanaka factors (c-Myc, SOX-2, OCT-4, KLF-4). iPSCs are grown in lab until they reach a large number & then they are induced to differentiate into killer T lymphocytes again. (Vizcardo *et al*., 2013)

Another way to produce the specific T cells is generating iPSCs from naïve T cells. First step is to harvest naïve T cells and then expose them to Yamanaka reprogramming factors. Reprogrammed killer T cells are grown & transduced with recombination receptors for tumor-specific antigens, which are known as CAR (Chimeric antigen receptor). Ultimately, these cells are induced to differentiate into T lymphocytes with the affinity for the selected tumor antigen present on cancer cells (**Fig. 4b**) (Themeli *et al*., 2013). Regeneration of T cells from the iPSCs is potential to create a mass of therapeutic T cells for cancer treatment.

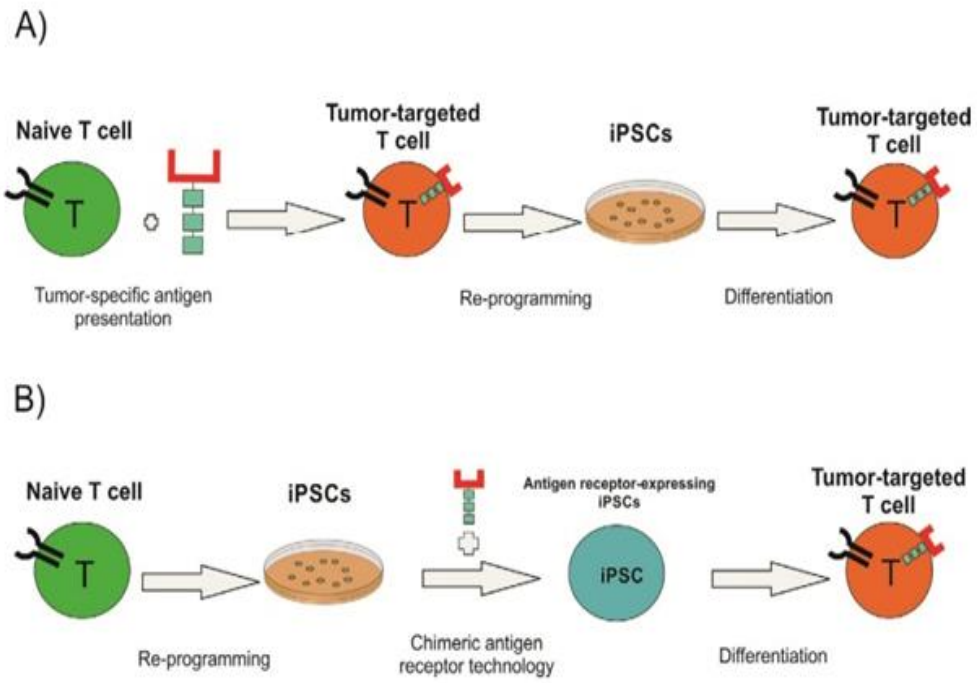

Figure 4.Generation of Tumor cell specific killer T cell.

A) Naïve T cells are primed with tumor antigens. Primed T cells are re-programmed into iPSCs to exploit the capability of self-renewing to get sufficient cells. iPSCs are finally differentiated into Tumor-targeting T cells.
B) Re-programming of Naïve T cells into iPSCs. After that, engineered to express the antigen receptors. Then iPSCs are expanded to enough numbers & induced to differentiate into T cells which are Tumor antigen-targeting T cells.(Nguyen *et al*., 2015)

Using Pluripotent Stem Cells as a Cancer Vaccine:

Of late, three standard cancer treatment methods are surgery, chemotherapy, radiotherapy. Main reason of failure of current treatment options is thought to be due to CSCs, which cannot be eradicated by traditional treatment procedures. CSCs are resistant to drugs; so, they could be resistant from radiation & conventional chemotherapy. This CSCs concept therefore implicates new approaches in treatment of

cancer therapy.

PSCs are valuable source which have large amounts of CSCs specific antigens. Conditioned medium of PSCs can provide an environment like the embryonic environment that promote the cancer management through differentiation therapy. Inactivated PSCs have been used against ovarian, lung, colon cancer in animal models (Li, Y., *et al*.,2009; Zhang *et al*.,2013). In immunosuppressive environment of the tumors, cancer cells have the ability to suppress the activation of immune cells through several mechanisms, including recruitment of immunomodulatory MSCs, myeloid derived suppressor cells (MDSCs), & regulatory T cells (Tregs), attenuation of MHC-I expression, and the use of PD-1/PD-L1 axis, cancer vaccines based on cancer specific antigens may educate the immune system against the cancer cells, so that it can encounters and eliminates them.

Main idea behind a cancer vaccine is to find out the optimal antigen & vaccine delivery system that can stimulate immune cells. As a result of these cancer vaccines, immune responses could be guided selectively toward cancerous cells of the patient while preventing normal cells from immune attack. The process by which immune system recognized & kills cancer cells is dependent on activation of antigen specific T cells by Antigen presenting cells (APCs) including dendritic cells, B cells, and macrophages. Induction of immunological memory by dendritic cells overcomes cancer cell plasticity and tumor recurrence in future. But, in most patients, functional DC is difficult to achieve because of chemotherapy or tumor's immunosuppressive state (Rami *et al*., 2016).

Cancer therapies based on DCs enhance the ability of immune system to recognize CSCs through presentation of surface antigens. Recently, DCs were generated from iPSCs (Senju *et al.*, 2011). Then DCs are loaded with cancer antigens by various ways and reinfused into patient (**Fig. 5**). Cancer antigens can be generated from tumor lysates (Yu *et al.*, 2004), apoptotic bodies (Labarriere et al., 2002), peptides (Rosalia *et al.*, 2013), tumor RNAs (Kalady *et al*., 2004), & tumor derived exosomes (Mahaweni *et al.*, 2013). Cancer antigens could be loaded onto DCs by nano-sized carriers. Of late, clinical study on glioblastoma have revealed that when patients were vaccinated with DCs transfected with mRNA derived from patient's own CSCs, an immune response triggered by vaccination were identified. In comparison with untreated patients, progression-free survival was 3 times longer in vaccinated patients.

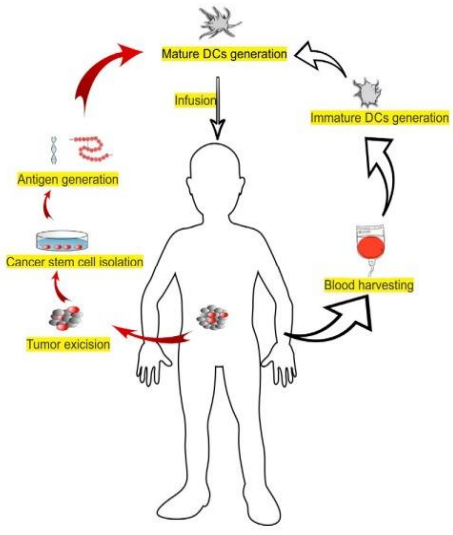

Figure 5. Cancer vaccination using cancer stem cell-derived antigen-primed dendritic cells.

At first, tumor is excised from the patient. Then CSCs are isolated from tumor biopsy and used to generate CSCs-derived antigens. Immature DCs are generated from peripheral mononuclear cells blood, or bone marrow cells. Then immature DCs are primed with CSCs-derived antigens or CSCs-derived mRNA to induce mature DCs presenting CSCs-derived antigens are infused into patients to eradicate CSCs. (Nguyen *et al.,* 2015)

Epigenetic reprogramming - Reversing cancer state to normal phenotype:

Pluripotency, reprogramming & malignant transformations are associated processes which are dictated by epigenetics (Moriguchi H *et al*., 2013). For example, in transformed lung fibroblast, epigenetic silencing of tumor suppressor gene p16 (CDKN2A) was reported as a cancer driven mechanism. The reprogramming successfully restored the p16 gene expression and it was also associated with elimination of epigenetic lesions associated with cancer (Hu K *et al*., 2011). Many other studies also suggest that the effects of cancer reduced after reprogramming & can also be used as a potential therapy for cancer management.

Several other studies showed contradictory results also. In a recent study, co-expression of both OCT-4/NANOG enhanced malignancy in lung adenocarcinoma and induces epithelial-mesenchymal transition (EMT). The aberrantly expressed pluripotency genes could enhance self-renewal capability to cancer cells. Mayo Clinic has discovered the reversing of cancer cells back to the normal state by epigenetic reprogramming. Kourtidis *et al*. (2015) highlighted the roles of p120 catenin & E-cadherin in exerting pro-tumorigenic activities of these proteins by balancing epithelial homeostasis in cells of normal phenotype. They have also identified PLEKHA7 as a specific marker of ZA (zonula adherens) which leads to suppression of growth of transformed cells. Ultimately, they discovered that an interaction of the ZA with microprocessor complex regulates a set of miRNAs which suppress cellular transformation and helps to maintain normal epithelial phenotype (Kourtidis *et al*., 2015).

Gene correction in iPSCs and autologous transplantation:

Molecular scissors like zinc finger nucleases (ZFN), Clustered regularly interspaced short palindromic repeats (CRISPR) & Transcription activator like-effector nuclease (TALEN) are important gene editing tools to conduct precise-genome editing including the correction of mutations which are associated with diseases (Urnov *et al*., 2010; Wu *et al*., 2014; Sander *et al*., 2014). Most of the cancers are caused because of mutation in a specific gene; that mutated gene of patient cells can be corrected through these genome editing tools. Corrected cells of patient can be converted into iPSCs which could be used further in autologous transplantation approach as a therapy in future. For example, mammary gland is the main target of breast cancer caused by the mutations in the BRCA1/ BRCA2 genes (Brody *et al*., 1998; Jiang *et al*., 2014). Treatment for breast cancer generally involves the combination of radiotherapy and chemotherapy, on many occasions which results in damaging or harming the mammary glands. The only way to restore the function of mammary gland is by regenerating the damaged gland. (Li *et al*., 2014)

But there are many limitations to the use of gene editing tools to precisely delete or modify cancer genes in patient as a therapy. Efficiency of gene targeting by using homologous recombination in iPSCs is very less. The utilization of viral vectors for gene modifications causes insertional mutagenesis which appears for the most critical problem in clinical translation.

Conclusion:

Despite multiple therapy attempts the cancer has not been defeated completely. Tumor cell heterogeneity, which causes differences in their biology, metabolism & signaling pathways as well as the effects of the microenvironment on normal cells are main challenges. Though, recent research demonstrates a wide variety of ways including engineered stem cells to combat cancer. Induced pluripotent stem cells have initiated a new era in the field of cancer cell biology and is the most promising technology.

Though, it is unclear if growing stem cells in the laboratory can induce any mutations that might cause disease later. Many studies have shown Mesenchymal stem cells (MSCs) promote the process of cancer metastasis (Halpern *et al*., 2011; Karnoub *et al*., 2007; Swamydas *et al*., 2013). Another remaining issue is the rejection of transplanted stem cells. It can also be difficult to find donors whose human leukocyte antigens closely match the patient's.

In the field of cancer, iPSCs have provided a double-edge sword that enables scientists not only to unravel the long mystery of epigenetic events contributing to cancer but also a renewable resource to develop a plethora of immune cells for immunotherapy.iPSCs are also easy to grow, proliferate indefinitely & contain the broad potential to form several different cell types.iPSCs are generated by insertion of genes by viruses into the genome. This raises the risk of creating mutations that could transform stem cells into cancer cells. More studies need to be performed to decide the long-term safety and efficacy of ex vivo expanded stem cells to utilize in cancer therapy engineering. The ample amount of knowledge that have been generated from this technological advancement may lead to better drug screening that will have more targeted effects and less toxicity. Thus, the iPSCs technology will accelerate the field of scientific research in finding new treatments for treating cancer.

Abbreviations:

iPSCs: Induced pluripotent stem cells; Sox2: Sex determining region Y; Klf4: Kruppel-like factor 4; c-Myc: Myelocytometosis; EMT: Epithelial-mesenchymal transition; DC: Dendritic cells; BRCA: Breast cancer; Oct4: Octamer-binding transcription factor 4; SCT: Stem cell transplant; MSCs: Mesenchymal stem cells; CSCs: Cancer stem cells.

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