Plasticity of cancer stem cell: origin and role in disease progression and therapy resistance

Running Head: Plasticity of cancer stem cells

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Abstract

In embryonic development and throughout life, there are some cells, which can exhibit phenotypic plasticity. Phenotypic plasticity is the ability of cells to differentiate into multiple lineages. In normal development, plasticity is highly regulated whereas cancer cells re-activate this dynamic ability for their own progression. The re-activation of these mechanisms enables cancer cells to acquire a cancer stem cell (CSC) phenotype- a subpopulation of cells with the increased ability to survive in a hostile environment and resist therapeutic insults. There are several contributors, which fuel CSC plasticity in different stages of disease progression such as a complex network of tumour stroma, epidermal microenvironment and different sub-compartments within tumour. These factors play a key role in the transformation of tumour cells from a stable condition to a progressive state. In addition, flexibility in the metabolic state of CSCs helps in disease progression. Moreover, epigenetic changes such as chromatin remodeling, DNA methylation could stimulate the phenotypic change of CSCs. Development of resistance to therapy due to highly plastic behaviours of CSCs is a major cause of treatment failure in cancers. However, recent studies explored that plasticity can also expose the weaknesses in CSCs, thereby could be utilized for future therapeutic development. Therefore, in this review, we discuss how cancer cells acquire the plasticity, especially the role of the normal developmental process, tumour microenvironment, and epigenetic changes in the development of plasticity. We further highlight the therapeutic resistance property of CSCs attributed by plasticity. Also, outline some potential therapeutic options against plasticity of CSCs.

Keywords: CSC plasticity, cancer heterogeneity, tumour microenvironment, cancer metabolism, therapeutic resistance, therapeutic options

1. Introduction

Cancer is a highly complex disease, displaying heterogeneity in different cancers and among cells within a single cancer [1]. The emergence of this complex process of tumour heterogeneity is always debatable and numerous cellular mechanisms *e.g.* metabolic switching, epigenetic alterations, deregulated signalling pathways *etc.* have been proposed to resolve the diversity between cancers and/or within cancer cells in a cancer [2]. In addition, plasticity (a process by which cancer cells gain the dynamic ability to switch from non-cancer stem cells to cancer stem cells and vice versa) has added further complexity to the highly discussed paradigm of tumour heterogeneity [3]. However, the plasticity of cancer stem cells (CSCs) could help in interpreting the heterogeneity observed in cancer [3]. CSCs, a subpopulation of cancer cells, associated with carcinogenesis, progression, resistance to therapy and cancer relapse, could directly switch between non-tumorigenic and tumorigenic cell states [4-5]. This reversible conversion capacity of CSCs attributes to protect themselves from chemo-radiotherapeutic insults [6-7]. Interestingly, this inter-conversion depends on the response to exogenous and endogenous stimuli and regulated by many factors including genetic evolution and phenotypic plasticity of CSCs [8, 9]. Cellular interactions and specific microenvironmental signals in tumour niche contribute to the process of CSC plasticity. Tumour microenvironment primarily provides autocrine and paracrine signals in gaining CSC plasticity and proceeds through the formation of a complex signalling network. This process is aided by the involvement of numerous factors including exosomes, tumour-stroma interactions, composition of extracellular matrix and many more, which varies upon time and space in tumour progression [6-7]. Furthermore, interactions of a complicated array of signals couple with gene expression to control signalling pathways. Consequently, changes in gene expression causes individual cells to expose a variety of phenotypic states, which in turn facilitate tumour growth, metastasis and the resistance to chemotherapy [6-7].

Metabolic change in CSCs is another key regulator of plasticity, which affects their emergence and persistence throughout disease progression. Heterogeneous metabolic phenotypes *i.e.* various levels of glycolysis and oxidative phosphorylation were noted in CSCs, which are directly linked to carcinogenesis [10].

In addition, epigenetic deregulations such as alterations in chromatin and DNA methylation, resulting in genetic damages, trigger cellular plasticity and thereby facilitate oncogenic cellular reprogramming [11]. Subsequent epigenetic changes, which are resulted from the interaction within the tumour niche, revamp cancer cell's phenotypes and properties, thus formulating tumour architecture, which in turn affects the cellular states at multiple stages of cancer progression [11]. Hence, in the context of understanding tumourigenesis and its pathogenesis, plasticity of CSCs is vital. In this review we, therefore, discuss how could plasticity of CSCs evolve and the role of metabolism, tumour microenvironment and epigenetic changes in the development of plasticity of CSCs. We further discuss the role of CSC's plasticity in therapy resistance and illustrate ways to target this highly menacing property of CSCs.

2. CSCs take over the developmental program of normal stem cell

Plasticity of cells is crucial for the successful development of multi-cellular organism, especially in human. Thus, it is not surprising that cancer cells hijack this mechanism from normal stem cell for their own development [12]. The underlying mechanisms of normal reprogramming could be seized by cancer cells for generating less differentiated and more stem-like cells (CSCs) with different phenotypic plasticity. Epigenetic alterations along with oncogenic driver mutations could induce this reprogramming in CSCs [13-14]. This reprogramming involves reactivation of developmental programs, which in turn changes the adaptation capacity of tumour cells. In normal cells, differentiation states are strongly

regulated through the activation and inactivation of transcriptional factors, which facilitate cellular plasticity during development [15]. Whereas in cancer, these factors coincide with those that contribute phenotypic plasticity as they are aberrantly activated (Figure 1). For instance, signalling pathways such as Notch and Wnt play prominent roles in cell fate switching, tissue patterning, and morphogenesis during normal development [15]. In cancer, these pathways can also contribute to the regulation of differentiation and self-renewal of CSCs in different molecular subtypes of cancer [15].

Phenotypic plasticity during tumour initiation is driven by the activation of developmental differentiation program, namely the epithelial-to-mesenchymal transition (EMT) [16]. EMT is a well-documented machinery of phenotypic plasticity in both normal and cancer cells. It plays a significant role in organogenesis during embryonic development, wound healing and cancer formation [17]. It is an essential ability of a cell to switch between epithelial to mesenchymal phenotypes during development [18]. For example, neural crest progenitor cells undergo EMT in the time of neural tube formation [19]. This process also occurs during embryonal development (gastrulation state) and formation of heart [16]. EMT is involved in undifferentiated or stem-like state, associated with the capacity of extended self-renewal and the acquisition of a stem-like genes expression [20]. However, some cancer cells undergo EMT whereas others do not, which could reflect intrinsic properties of their cell of origin. For example, inter-follicular epidermis and hair follicle tumour initiating cells shows distinct EMT properties [20]. Nevertheless, many of the molecular mechanisms of EMT in cancer are like EMT in normal cellular process such as in wound healing [5]. Other mechanisms contributing to cellular plasticity include activation of key-genes' transcription factors *e.g.* Snail, Zeb, and Twist families, which manoeuvre the transcriptional networks for de-differentiation by mediating specific interaction with DNA, thereby regulating gene expressions [21-23]. These transcriptional regulators not only regulate the transcription

process in the normal development, but also play a significant role in cellular plasticity of CSCs. For instance, Zeb 1 regulates stemness, colonization capacity and phenotypic/metabolic plasticity of pancreatic adenocarcinoma driven by the activation of oncogenic *Kras* and deletion of *p53* [24]. In addition, it promotes stem-like tumourigenic phenotype and resistance to MAPK inhibitors in melanoma stem cells [24]. Table 1 represents the factors involved in normal development and contributed in plasticity of CSCs.

In addition, tumour plasticity follows the normal developmental history of organs as they can gain cell fates involved in developmentally related neighbouring organs [25]. Defects in transcription factors programming are associated with embryonic cell-fate specification, which causes formation of tumour plasticity characterized by the acquisition of alternative cell fates of adjacent organs [25]. For example, downregulation of lineage-specifying transcription factor NKX2-1 from murine alveolar epithelium (not airway epithelium), resulted in the conversion of lung cells to gastric-like cells [25]. Similarly, in non-small cell lung carcinoma, loss of NKX2-1 causes accumulation of cancer like features in various gut tissues [25]. Therefore, the developmental programme appears to be a critical mechanism by which CSCs gain their ability to self-renew and maintain their plasticity.

3. Extrinsic and intrinsic factors involved in plasticity of CSCs

3.1 Role of tumour microenvironment in plasticity of CSCs

The tumour microenvironment is an important factor that contributes to the plasticity of cancer cells, including CSCs [26]. It is a complex network, consisting of tumour stroma, epidermal microenvironment and different sub-compartments within the tumour [26]. Many cellular and non-cellular factors from this microenvironment contribute to the transformation of tumour cells and may protect them from therapeutic insults [27]. It was demonstrated that CSCs, which are stimulated to gain plasticity as differentiated cancer cells could be

reprogrammed in response to specific environmental signals, thereby reinitiating proliferation capacity and CSC-like features [27-28]. Accordingly, when cancer cells obtain specific signals from their microenvironment, they fuel the mechanism of plasticity, causing shift from an unstable "static" hierarchical condition to a reprogrammed state [3]. Thus, the interactions with tumour microenvironment corroborate malignant behaviour of tumour cells and drive the mode of phenotypic plasticity of cancer cells. For instance, the interconnection between CSCs and non-CSCs neoplastic cells in stroma of pancreatic, breast, and colon cancer, where stromal cells secrete signalling factors, which are received by epithelial cells, causes signalling cascade to orchestrate an epithelial to mesenchymal transition [29-31]. Furthermore, this interaction triggers the generation of CSCs phenotype by the activation of paracrine Nodal/Activin signalling cascade [32-33].

The mutual communication between CSCs microenvironment and immune niche can induce cellular plasticity (Figure 2). For example, pro-inflammatory mediators such as tumour necrosis factor (TNF) and interleukin-6 (IL-6), secreted by immune cells of the tumour microenvironment, can regulate the phenotypic changes of CSCs [34]. This cytokine-driven plasticity of CSCs have been noted in breast carcinoma, melanoma, and lung carcinoma, where TNF and IL-6 can alter the differentiation state of CSCs by upregulating mesenchymal genes and promote EMT-type switch [3]. Moreover, in the absence of necessary factors required to revitalize self-renewal process, CSCs secrete IL-6 in order to attract mesenchymal stem cells (MSC), which in turn promote cancer cell stemness by upregulating of NF-κB [28, 35-36]. Additionally, CSCs seize normal stem cell niches formed by MSCs for transforming surrounding cells to support CSCs to colonize the new niche [35]. Therefore, in such a microenvironment, tumour cells gain the capability of controlling immune response by facilitating the expansion of tumour-associated macrophages, tumour-associated neutrophils, myeloid-derived suppressor cells and dendritic

cells (Figure 2). Subsequently, tumour-associated macrophages produce TNF-α and TGF-β, which in turn stimulate NF-κB or TGF-β-dependent induction of EMT and stemness pathways, resulting in further augmentation of self-renewal and plasticity of CSCs.

Hypoxia, another phenomenon of tumour microenvironment, can induce the transformation of non-CSCs to CSCs, thereby triggers plasticity of CSCs. Hypoxic stress in inconsistent tumour microenvironment can induce metabolic, epigenetic and phenotypic reprogramming of the cells [37-38]. Consequently, hypoxia can promote the capacity of a cell to switch from its original cellular state to a distinct one [39]. HIFs (Hypoxia-inducible factors) with pro-angiogenic and inflammatory factors such as VEGF, or TGF-β could play important regulatory roles in hypoxia-induced CSC plasticity [40]. For example, HIF-2α causes the release of angiogenic factors to promote the acquisition of CSC-like phenotype in glioblastomas [41]. In triple-negative breast carcinoma, hypoxia induces the acquisition of cancer stem-like phenotypic plasticity via upregulation and activation of STAT3 (signal transducer and activator of transcription-3) [42].

Biomechanical forces such as hydrodynamic shear stress might be an important microenvironmental factor, which leads to the generation of cancer stem-like cells or tumour initiating potential in cancer [43]. It was noted that shear stress facilitates the conversion of circulating tumour cells into distinct tumour initiating cells in blood circulation by enhancing plasticity via EMT through inhibiting ERK and GSK3β activities [43]. Also, in the presence of oxygen and nutrient gradient, melanoma spheroids separated themselves into a continuously proliferating subpopulation in the periphery, whereas a subpopulation of G1-arrested cells in the centre [44]. Similarly, in human melanoma xenografts in mice, it was found that cells located near the blood vessels are going through the cycling process, whereas tumour cells in the centre are quiescent. Furthermore, when these two populations were cultured in a 2D culture plate, within 24 hours, G1arrested cells re-entered their cell cycle and

not surprisingly, they became indistinguishable from the subpopulation of peripheral cells. Thus, these results indicate the phenotypic cellular plasticity model is influenced by specific environment. Therefore, CSC's function and plasticity may be affected by specific microenvironmental signals and cellular interactions originating from the tumour niche.

3.2 Metabolic adaptation fuels plasticity of CSCs

Metabolic plasticity is crucial for cancer cell adaptation. The complexity of CSC metabolism and their phenotypic behaviours are important prospects of cancer research. Normal stem cell depend heavily on oxidative phosphorylation (OXPHOS) for their energy, and a non-CSC cancer cell is primarily glycolytic, whereas CSC exhibits a solitary metabolic flexibility [45]. CSCs possess distinct metabolic profiles as they are endowed with elevated glucose consumption, lactate synthesis, and ATP production when compared to non-CSCs [46]. In addition, metabolic plasticity exists in CSCs of certain cancer subtypes, as well as can be found within the same cell type, thus contributing to metabolic heterogeneity [46]. Being a less differentiated phenotype, it is believed that CSCs depends mainly on glycolysis compared to their more differentiated counterpart [47]. However, to maintain homeostasis as well as tumour growth, CSCs can shift between OXPHOS and glycolysis. In various types of cancers such as in glioblastoma, lung carcinoma, colon carcinoma, osteosarcoma, ovarian carcinoma and breast carcinoma, CSCs strongly prefer the glycolytic pathway rather than more differentiated cancer cells [48-52]. Furthermore, stimulation of glycolysis by up-regulation of glycolytic enzymes (*e.g.* GLUT1, HK-1, and PDK-1) is necessary for the longevity of CSCs [53-55]. In addition, reprogramming the metabolic shift from OXPHOS to glycolysis was shown to enhance the CSC properties [10]. Moreover, glycolysis was found to be the preferred metabolic program in radio-resistant nasopharyngeal carcinoma's CSCs and CD133+ CD49f+ CSCs in hepatocellular carcinomas [13, 46]. The underlying

mechanism of CSC's metabolic switch towards glycolysis was evident by significant roles of STAT3 in cellular metabolism of STAT3 dependent CSCs [56]. Active STAT3 (Tyr 705) induces a metabolic switch to aerobic glycolysis and downregulates mitochondrial activity in primary fibroblasts and other STAT3-dependent CSCs [56]. On the contrary, a recent study demonstrated enhanced glycolysis as well as increased OXPHOS in CSCs [57]. However, higher rates of oxygen consumption and increased mitochondrial functions suggested a preference of OXPHOS in many types of cancers [58-60]. For example, CD133+ CSCs in glioblastoma and pancreatic adenocarcinoma, low reactive oxygen species quiescent leukaemia, side population (the population of cells which exclude dye and possess CSC property) of lung carcinoma, and side population of breast carcinoma prefer mitochondrial oxidative metabolism as the energy production process rather than glycolysis [58-60]. In CD133+ glioblastomas, insulin-like growth factor 2 mRNA binding protein (IMP2) maintains the metabolic switch to OXPHOS by directly interacting with several mitochondrial complex genes and regulating the expressions of stemness markers, including CD133, Sox2, Oct4 and Nanog [61]. Interestingly, in addition to the glycolytic and classical OXPHOS phenotypes, CSCs also rely on mitochondrial FAβO for ATP and NADPH generation (Figure 3). Thus, it is likely to occur that CSCs have adopted a relatively plastic metabolic state and can adjust to the settings in which the cells reside.

Accumulating evidence suggests various metabolic phenotypes in a tumour depending on their locations. Different compartments may be actively proliferating regions of the tumour, with adequate levels of oxygen, hypoxic areas of the tumour, and in a distant metastatic site [45, 62]. For example, in normoxic condition where stemness has been associated with increased production of glycolytic enzymes, CSCs rely on glucose pathway along with mitochondrial pathway (Figure 3). Increased membrane potential, mitochondrial mass and mitochondrial fatty acid oxidation (FAO) for generation of ATP and nicotinamide

adenine dinucleotide were noted in CSCs with sufficient levels of oxygen [62]. However, in hypoxic condition, where oxygen supply is deprived, hypoxia-inducible factors 1 (HIF-1) improves the up-regulation and activity of glycolytic proteins such as GLUT1, GLUT3 and various isoforms of glycolytic enzymes [63]. Moreover, in the metastatic niche, CSCs increases the utilization of extracellular catabolites, such as pyruvate, lactate, glutamate, glutamine, alanine, or ketone bodies [64]. On the other hand, in nutrient-deprived condition, CSCs maintain a quiescent state and the necessary energy acquired through autophagy [65-67]. Therefore, putting aside the controversy about the metabolic phenotype of CSCs, metabolism is not only a key player but also a regulatory instigator of CSC's plasticity (Figure 3).

3.3 Epigenetic mediated regulation of plasticity in CSCs

Epigenetic mechanisms are the molecular processes that affect cell's behaviour via changing genes expression without genetic alterations [11]. They are primarily interceded by alterations in chromatin structure and DNA methylation pattern, which in turn confer genes differentially compatible for transcription [11]. It was demonstrated that various epigenetic changes in CSCs increase cellular plasticity and allow reversible transitions between different phenotypic states [11]. In addition, during initial carcinogenesis, changes in chromatin and DNA methylation resulting from epigenetic alterations trigger cellular plasticity and facilitate oncogenic cellular reprogramming. Epigenetic changes induced by chromatin or chromosomal rearrangement, are very common and crucial to maintaining the plasticity of CSCs [68]. For example, differentiated cancer (*e.g.* CD44 low) cells re-acquire self-renewal ability and reverse to a CSC (CD44 high) state by changing epigenetic and genetic makeup stimulated by TGF-β in breast carcinoma [68]. Similarly, in melanoma, chromatin states can influence cancer cell plasticity [69]. It was found that a subpopulation of cells in melanoma

is required for the continuous tumour growth and was distinguished by the expression of the histone demethylase JARID1B. This population transiently acquire stemness property depending on the tumour context [70]. On the other hand, histone acetylation, mediated by histone acetylases (HATs), regulates histone activity and increases gene expression by post-translational modification (Figure 4). DNA methylation, another post-translational modification, favours CSCs formation and maintenance [71]. Importantly, proteins, (*e.g.* DNA methyltransferases, methylcytosine dioxygenases) associated with DNA methylation, have been identified as the drivers of CSC generation by controlling the nature of CpG dinucleotide formation (Figure 4). For example, in leukaemia and many solid tumours, changes in DNA methylation pattern leads to generation of CSCs [72-73].

4. CSCs plasticity and therapy resistance

There are several useful chemotherapeutic options exist for patients with cancer. However, development of therapeutic resistance is the major drawback and cause of treatment failure and disease recurrence. A few underlying mechanisms are associated with plasticity derived therapy resistance of CSCs [74-76]. Therapy resistance property of CSCs depends on the interplay between microenvironmental signals, metabolic adaptation, expression of transcription factors and epigenetic alterations *etc.*, which in turn contribute to the plasticity of CSCs [77-79]. It was noted that cells exhibiting CSC or mesenchymal-like phenotype show enhanced resistance to conventional chemotherapeutic agents when compared with more differentiated or epithelial-like cancer cells [80]. These therapy-resistant mesenchymal-like cells populations are responsible for cancer relapse after treatments [80]. In addition, the augmentation of CSCs phenotype after therapy could be due to the increased symmetric division of these cells, and due to the shift of non-CSCs to the CSC state. For example, in glioblastoma exposure of differentiated non-CSCs to

temozolomide (TZM) resulted in re-expression of stem cell markers such as SOX2, OCT4 and Nestin, which in turn lead further expansion of CSCs [81]. Therefore, acquisition of therapeutic resistance may be a dynamic and reversible process, and under therapeutic pressure, cells can switch between the drug-resistant and drug-sensitive states. Also, resistance against therapies is gained through promoting the acquisition of a de-differentiated state by increasing the expression of stemness-related genes [82-83].

Additionally, trans-differentiation is another mechanism of cellular plasticity associated with resistance to therapy [84-85]. For instance, in advanced prostate adenocarcinoma, androgen deprivation therapy can ultimately lead to resistance and progression of aggressive phenotypes, which manifests phenotypic and molecular characteristics of neuroendocrine differentiation [84-85].

Tumour microenvironment could add fuel to the therapy resistance property of CSCs [86-88]. Local signals from tumour microenvironment influence the generation of several drug-tolerant phenotypes such as EMT and CSC-like states in region-specific manner in tumour. For example, TGFβ, IL-6, exosomes, and many other cancer-associated fibroblasts (CAF)-secreted cytokines as well as growth factors have been identified to push the formation of drug-resistant EMT phenotypes in the heavily localized CAFs invasive tumour front [86-88]. Likewise, hypoxia can induce therapeutic resistance by creating a signalling network favourable to drug-tolerant EMT states [89]. Therefore, in the notion of therapy resistance, plasticity of CSCs plays a pivotal role. To ensure the proper therapeutic outcome in clinical settings, newly designed drug must have the ability to escape or diminish this dynamic property of CSCs.

5. Therapeutic options against plasticity of CSCs

Development of treatment strategies targeting directly the dynamic and plastic behaviour of CSCs can have attractive therapeutic potential. From the therapeutic standpoint, plasticity of cancer cells always creates problems as the presence of multiple phenotypes within a single tumour always holds the risk that a given therapy will fail to kill some of the tumour cells [90]. Therefore, greater efforts should be taken, firstly by understanding the origin of diversity of the tumour and then, by exploiting that knowledge to design novel therapy. Table 2 represents the potential therapeutic options targeting plasticity of CSC.

Presently, several clinical strategies have been proposed that could be effective against plasticity of CSCs. Firstly, therapeutics blocking or reversing de-differentiation of CSCs as they could be generated de novo by dedifferentiation of non-CSCs, which prevent cancer cells from becoming metastatic and developing drug-resistant phenotype [90]. Secondly, therapeutics could be beneficial to patients by neutralization the factors associated with the promotion of EMT. Thirdly, treatment can be targeted by blocking the signalling pathway used by EMT cells to evade, survive in the circulation, or resist therapy against cancer. Thus, it is critical to destroy cancer cells that undergo EMT to not only block or reverse that process. Interestingly, various approaches have already been taken to combat signalling pathways that induce EMT such as TGF-β, STAT3, miR-210 [91-94]. Importantly, additional research might be required to justify the efficacy of these strategies with respect to their specificity to CSCs in vivo. Subsequently, as cellular plasticity is involved with partial EMT, it will be necessary to test the efficacy of these new strategies targeting hybrid epithelial/mesenchymal cells [95].

In addition, hedgehog signalling plays a significant role in cancer-associated fibroblasts (CAFs), thereby acting as a novel mediator of CSC plasticity and opens up a doorway of exciting new therapeutic target in triple-negative breast carcinoma [96].

Hedgehog ligand produced by neoplastic cells in mouse models, reprograms CAFs to confer a supportive niche for the accumulation of chemo-resistant CSC phenotype via FGF5 expression [96]. Accordingly, stromal treatment with smoothened inhibitors (SMOi) impedes CSC markers expression and sensitizes tumour cells to docetaxel, leading to markedly improved patients' survival and reduction of metastatic burden of cancer [96]. In a phase-I clinical trial (NCT02027376), 3 out of 12 patients with metastatic triple-negative breast carcinoma obtained clinical benefit from combination therapy with SMOi, Sonidegib and docetaxel [96].

Targeting metabolic plasticity of CSCs has become an emerging area to effective elimination of CSCs. To inhibit glycolysis in CSCs, glucose transporter and glycolytic enzymes such as GLUT1-4, hexokinase1-2, pyruvate kinase M2, and lactate dehydrogenase have been proposed as attractive targets [97-99]. Moreover, mitochondrial metabolism could be a useful target to eliminate OXPHOS phenotype of CSC in numerous cancers [60, 100-101]. Targeting CSCs through inhibition of mitochondrial biogenesis and OXPHOS are currently under investigation for cancer treatment. For instance, salinomycin, erythromycins, tetracyclines, and glycylcyclines have already demonstrated efficacy in eradicating CSCs via blocking the plasticity of CSCs [101-104]. Another compound, metformin, an inhibitor of OXPHOS complex-I has demonstrated anti-cancer activity by reducing mammosphere formation, in vivo tumour growth, and inducing apoptosis in pancreatic CSCs via preventing metabolic switch to glycolysis [60, 105-106]. Since CSCs can also expose intermediate glycolytic/ OXPHOS phenotype, therefore treatment option targeting this intermediate phenotype could be effective in eradicating CSCs. Interestingly, mitochondrial reactive oxygen species (ROS) inducer such as menadione could prevent or reverse glycolytic/ OXPHOS phenotype [101]. Dual mechanism of menadione inhibition of Complex-I, and induction of mitochondrial ROS points out the significant efficacy of multi-modal targeted

therapy. Moreover, inhibiting mitochondrial respiration not only induces apoptosis in pancreatic CSCs with OXPHOS phenotype but also effectively eliminates primarily glycolytic breast and nasopharyngeal CSCs [60, 107].

Another potential target is the inhibition of adaptive mechanism of CSC. It was noted that under heterogeneous environmental condition such as hypoxia, glucose deprivation, and low pH, CSC rapidly transits their metabolism and this adaptive metabolic switch by CSCs play a pivotal role in cancer metastasis or chemo-resistance [108]. There are several factors or enzymes involved in the metabolic adaptation of CSCs, which are sensitive to specific therapeutic actions. For example, HIF1-2 alpha is a key enzyme for metabolic adaptation in hypoxia and is associated with angiogenesis, metastasis, and cell survival [108]. Interestingly, there are some potential compounds such as TH-302, a nitroimidazole prodrug of the DNA alkylating agent in combination with doxorubicin, has been found effective against HIF in soft tissue sarcoma [109]. Another enzyme pyruvate dehydrogenase kinase 1 is enriched in breast CSCs and is critical for metastasis in hypoxia. Pyruvate dehydrogenase kinase 1 regulates the metabolic transition in hypoxia via controlling the amount of acetyl-CoA, which is oxidized in the mitochondria to produce energy in the TCA cycle [110]. Anti-cancer agent from Cinnamomum cassia Blume targets pyruvate dehydrogenase kinase 1 and induces mitochondria-mediated apoptosis in lung carcinoma stem cells [111].

Conventional chemo-radiotherapies are not effective against quiescent, slowly (or non-) dividing CSCs, thereby become resistant and subsequently repopulate the tumour [112]. Interestingly, it was found that a compound named thapsigargin targets highly plastic CSCs in a proliferation independent fashion and can thus effectively target quiescent cells [113-114]. However, thapsigargin is not selective for cancer cells, but recent efforts to modify it as a tumour-targeted pro-drug have greatly improved the specificity profile and undergoing phase II trials (NCT01056029) [115].

Epigenetic regulators, another potential class of therapeutic targets, could be conducive in regulating cell state plasticity [116-117]. For example, histone-deacetylase inhibitors (*e.g.* suberoylanilide hydroxamic acid, abexinostat) have been shown to promote differentiation of breast cancer cells and diminish the number of CSCs within cancer [116-117]. Moreover, a key feature of epigenetic mechanisms is their inherent reversibility, which helps cancer cells to gain cellular plasticity. This dependence of CSCs on epigenetic regulators offers an opportunity to target their self-renewal capacity. Overall, the concept of CSC plasticity is new and aiming drivers of plasticity mechanisms carries the future promise. Thus, further studies to identify and target drivers of CSC plasticity are imperative.

6. Concluding remarks

The present review discusses the critical insights regarding the plasticity of CSCs, emphasizing the mechanism through which CSCs acquire the phenotype. The phenomena such as program development, metabolic adaptation, epigenetic changes attribution in CSC's plasticity are highlighted. It is observed that phenotypic plasticity is directly associated with cellular origins of cancer as well as the progression of cancer and response to therapy. Moreover, it is important to apply the principles of targeting phenotypic plasticity as an anticancer target. If so, they may give rise to unexpected vulnerabilities that can be exploited to target cancer cells. Relevant factors driving the switch from hierarchy to plasticity are getting the attention to inhibit the plastic behaviour of cancer cells, especially of CSCs. In that case, inhibition of deregulated transcription factors, that regulate the normal cell differentiation as well as CSC, might be an attractive option. Therefore, targeting the sources for phenotypic plasticity in cancer cells, for instance, suppression of oncogenes and/or modification of tumour microenvironment can contribute to the reduction of CSC's plasticity. However, it is clear that CSCs occupy their plasticity by different mechanisms such as

microenvironmental signals, metabolic adaptations, epigenetic alterations *etc*. Since tumour cells maintain a network of changes in case of their progression, therefore targeting or inhibiting one property could boost others. Thus, to inhibit or stop plastic behaviour of CSCs focus should be set on combined targets or more directed therapies that could aim for more than one property of CSCs at a time.

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Figure legends

Figure 1: Development of plasticity or heterogeneity of CSCs. CSCs take over normal development process of cells. In normal cells, plasticity is strictly regulated by regulated interplay between activation and inactivation of transcription factors, signalling pathways, epigenetic check. In the case of cancer, cells deregulated transcriptional activation, signalling pathways, and abnormal epigenetic alterations result in the generation of plasticity.

Figure 2: Roles of tumour microenvironment in the generation of CSC plasticity. Cancer-associated fibroblast, immune cell and inflammatory cell promote CSC plasticity by various mechanisms. Tumour microenvironmental factors like hypoxia and hydrodynamic shear stress also contribute to the generation of CSCs plasticity. Straight-line indicates activation and truncated line denotes inhibition.

Figure 3: Metabolic switch and plasticity of in CSCs. Compared to normal cancer cells, CSCs maintain various metabolic phenotypes depending on the environmental stimuli and the supply of nutrient. In normoxic condition CSCs maintain combined (glycolysis+ OXPHOS) phenotype, whereas in hypoxia CSCs shift them towards glycolysis by HIFs mediated upregulation of GLUT1 and GLUT2. In nutrient deprivation state, CSCs holds a quiescent phenotype where the energy comes from the autophagy.

Figure 4: Roles of epigenetic alterations the plasticity of CSCs. Several histone modifications like acetylation, phosphorylation and methylation result in an open or closed chromatin structure, which in turn activates or represses gene expression and subsequently causes self-renewal and CSCs plasticity. Aberrant activation of chromatin remodellers like

SWI/SNF, ISWI also promotes the plasticity of CSCs. In addition, uncontrolled activation or inactivation of methylation of DNA cytosine can give rise the plasticity to CSCs.

Table 1: Factors associated with the plasticity of CSCs

Genes/Proteins/TFs	Functions	Cancer	Reference
PTEN	Loss of PTEN expression in prostate epithelial leads to increased plasticity of tumour cells	Prostate cancer	[3]
MiTF	Regulates whether the cell will differentiate, proliferate, or become quiescent with increased migratory behaviour.	Melanoma	[7]
Slug/Snai2	By repressing PUMA it inhibits cell death and upon cancer therapy	Hematopoietic cancer	[34]
Snai1	Activation of Perk kinase promoting therapy resistance through Nrf2 activation	Pancreatic cancer	[17, 37]
Nkx2.1	Loss of Nkx2.1 leads to the acquisition of gut fates	Mucinous adenocarcinoma	[38]
Myc	Overexpression of myc is the main driver of stemness and glycolytic flux	Breast, Nasopharyngeal and Hepatocellular carcinomas.	[69, 80]
STAT3	Causes metabolic switch to aerobic glycolysis and downregulates mitochondrial activity in primary fibroblasts	Glioblastoma, Breast and Intestinal cancer.	[81]
IMP2	Induces metabolic shift to OXPHOS by directly interacting with mitochondrial genes and also regulates the stemness markers including CD133, Sox2, Oct4 and Nanog.	Glioma	[89]
MCL1	Along with myc promote chemotherapy resistant CSC via the regulation of mitochondrial OXPHOS	Breast cancer	[97]
VEGF	Induces hypoxia related invasiveness	Pancreatic cancer	[130]
MMP3	Induce hypoxia related invasiveness	Pancreatic cancer	[130]
Oncostatin M	Promotes epithelial–mesenchymal plasticity through STAT3-SMAD3/TGF-β signaling.	Pancreatic adenocarcinoma	[173]

DHHC protein family	DHHC protein family promote CSCs plasticity in low oxygen condition and under less nutrient supply and expression varies in different subsets of glioblastoma.	Glioblastoma	[174]
MIF-1	Controls cancer cell plasticity by conversion of CD138- to CD138+ cells.	Multiple myeloma	[175]
HIF-1 and -2α	HIF-1α promotes the expression of stem cell-associated transcription factor Oct4	Breast cancer,	[63]
		Glioblastoma	[178]
	HIF-2α facilitates the release of angiogenic factors and promotes acquisition of a CSC-like phenotype		
GRHL2	Positively correlates with E- cadherin and CD133 expression and regulates epithelial plasticity along with stemness	Pancreatic cancer	[179]
KLF4	promotes a less differentiated state characterized by enhanced ECM production that establishes a pro-metastatic niche phenotypically-switched perivascular cells	Melanoma, Rhabdomyosarcoma	[180]
OLIG2	Plays critical role in the maintenance of tumour propagating neurospheres or self-renewal withTrp53/Pten deletions /PDGFB overexpression.	Glioblastoma	[181]
PIK3CA	Mutated PIK3CA induces reprogramming of lineage restricted progenitors to a multipotent stem-like state in breast tumour initiation	Breast cancer	[182]
FOXC2	Facilitates NE trans-differentiation as well as resistance to enzalutamide (ADT) and docetaxel.	Neuroendocrine prostate cancer	[183]
MLL5	Induces cell plasticity by repressing proneural differentiation of nonstem cancer cells	Glioblastoma	[184]

UNC5A	Loss of UNC5A expression could result in ERα-positive luminal cells acquiring basal features including the expression of ΔNp63, SOX2, and EGFR	Breast cancer	[185]
SOX9	A key regulator of epithelial cells proliferation and acquiring properties of basal stem cells; to the induction of EMT, the deposition of extracellular matrix and changes in cytoskeleton and adhesion. All the functions lead to the plasticity of cancer cells.	Prostate cancer	[186]
DRD2	Participates hypoxia related transcriptomic and metabolic plasticity	Glioblastoma	[187]
Brca1	Contributes to CSCs related heterogeneity by dysregulated lineage restriction	Breast cancer	[188]
Pik3ca	Activates multipotent genetic program in lineage-restricted mammary gland populations	Breast cancer	[189]
Zeb1	Maintains phenotypic/metabolic plasticity by activation of oncogenic Kras and deletion of p53	Pancreatic cancer	[32]
	Promotes stem-like phenotype and resistance to MAPK inhibitors	Melanoma	[33]
	Increases tumour propagation and cell plasticity through repression of miR-200 family and interaction with YAP	Pancreatic and Colorectal carcinoma	[190]
Twist1	Represses differentiation by activation of MAPK pathways	Melanoma	[36][191]
	Promotes therapy resistance by activating therapy resistance		

Smarce1	Drives invasiveness by partial EMT in early stage in situ tumours	Breast cancer	[192]
Jmjd3	Increases tumour-initiation through deposition of active histone mark on Snai2 promoter	Hepatocarcinoma	[193]
Imp3, Sirt2	Stabilizes Snai2 transcripts	Breast cancer	[194]
Taz	Induces plasticity and stemness in mammary epithelial cells	Breast cancer	[195]
	Interacts SWI/SNF complex to mediate cellular plasticity		
Dnmt3a, Dot1l	Mutation causes the loss of cell-cycle regulators and lineage-specific genes	Breast cancer	[196]

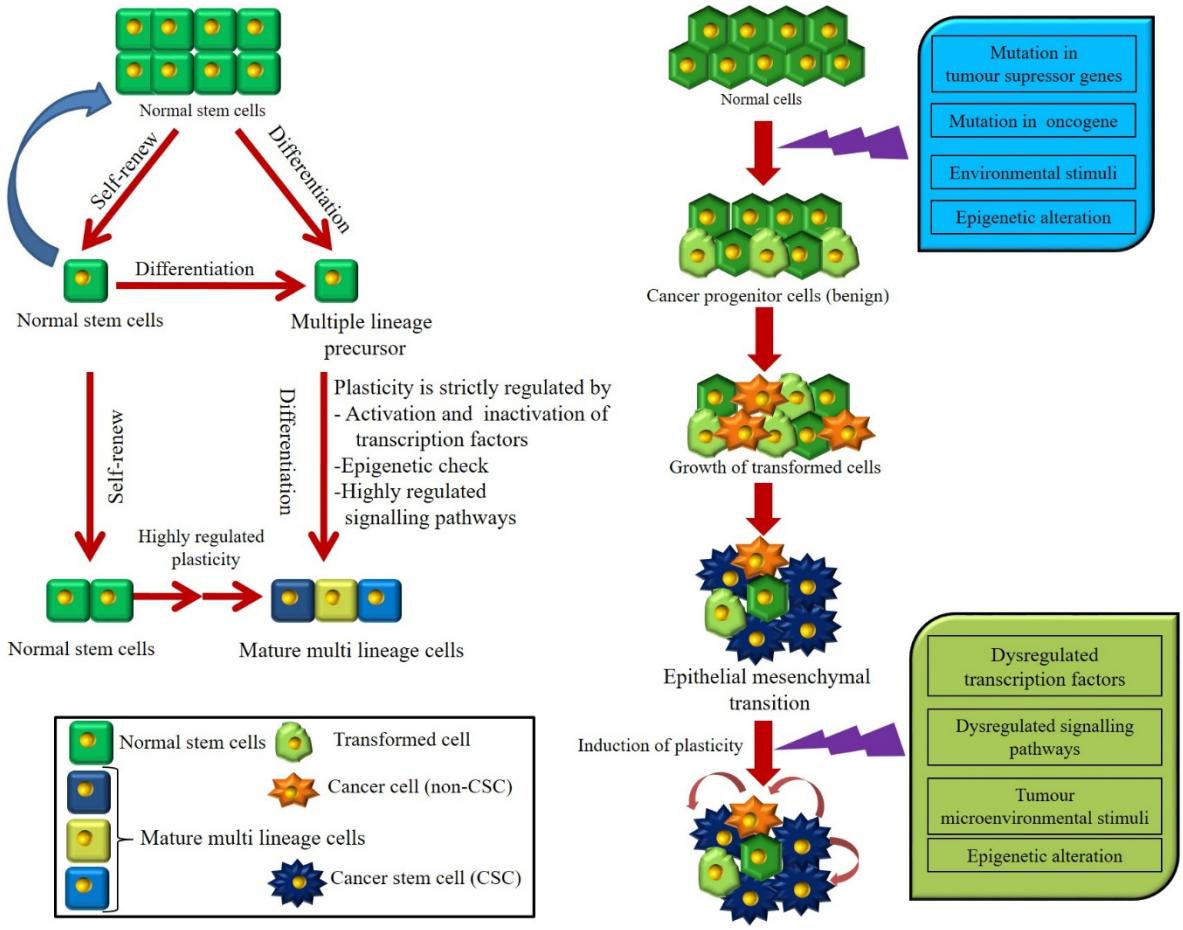
Abbreviation: Forkhead transcription factor (FOXC2); Microthalamia associated transcription factor (MATF)

Table 2: Available therapeutic options against CSC plasticity

Genes/synthetic compounds/natural compounds	Functions	Cancer	Reference
ATOH1	Overexpression of ATOH1 induced differentiation and reduced tumourigenicity both in vitro and in vivo	Gastric cancer	[131]
Galunisertib (LY2157299)	Inhibits TGFβ receptor kinase to prevent signal transduction mediated CSC growth	PDAC (NCT01246986), HCC (NCT01373164), Glioma (NCT01220271), Glioblastoma(NCT0217838)	[135]
STA-21	Inhibits STAT3-SH2 domain dimerization	Breast cancer	[137]
LLL-3	Inhibits STAT3-SH2 domain dimerization	Glioblastoma	[137]
Salinomycin	Inhibits the growth of mesenchymal phenotype of tumor cells	Breast cancer	[138]
Sonidegib	Downregulates CSCs markers expression and also sensitizes to docetaxel	Triple negative cancer	[141]
Menadione	Prevents glycolytic/ OXPHOS phenotype by ROS	Pancreatic cancer	[88, 152]
TH-302	Inhibits HIF in combination with doxorubicin	Sarcoma	[154]
Cinnamomum cassia Blume	Induces apoptosis by targeting pyruvate dehydrogenase kinase	Lung carcinoma	[156]
Thapsigargin	Selectively targets quiescent CSCs	Many solid tumors	[160]
Suberoylanilide hydroxamic acid	Induces cancer cell differentiation by inhibiting histone deacetylase	Breast cancer	[161]
Abexinostat	Induces cancer cell differentiation by inhibiting histone deacetylase	Breast cancer	[162]
TCTP	Repeals the malignancy through partially recovering the function of the P53/MDM2 axis	Breast cancer, Lymphoma	[168-170]
Metadherin	Facilitates MET by suppressing Twist1 and	PDAC	[197]

	contributes to anoikis resistance.		
Sorafenib	Decreases colony outgrowth, tumoursphere formation, ALDH1 activity, and tumour-initiating capacity by inhibiting downstream multikinase	Pancreatic cancer	[198]
5-Flurouracil	Targeting JNK signaling to increase chemotherapy sensitivity of CSC	Pancreatic cancer	[199]
Gemcitabine	Targeting JNK signaling to increase chemotherapy sensitivity of CSC	Pancreatic cancer	[199]
Metformin	Diminishes cell growth and proliferation by pro-oncogenic pathway (NF-κB and HIF-1α) mediated inhibition of pro-inflammatory cytokines like TNF-α and IL-6, as well as angiogenic cytokine VEGF	Pancreatic cancer	[200]
	Inhibits the CSC metabolic switch from OXPHOS to glycolysis	Pancreatic cancer	[88]

PDAC: Pancreatic ductal adenocarcinoma



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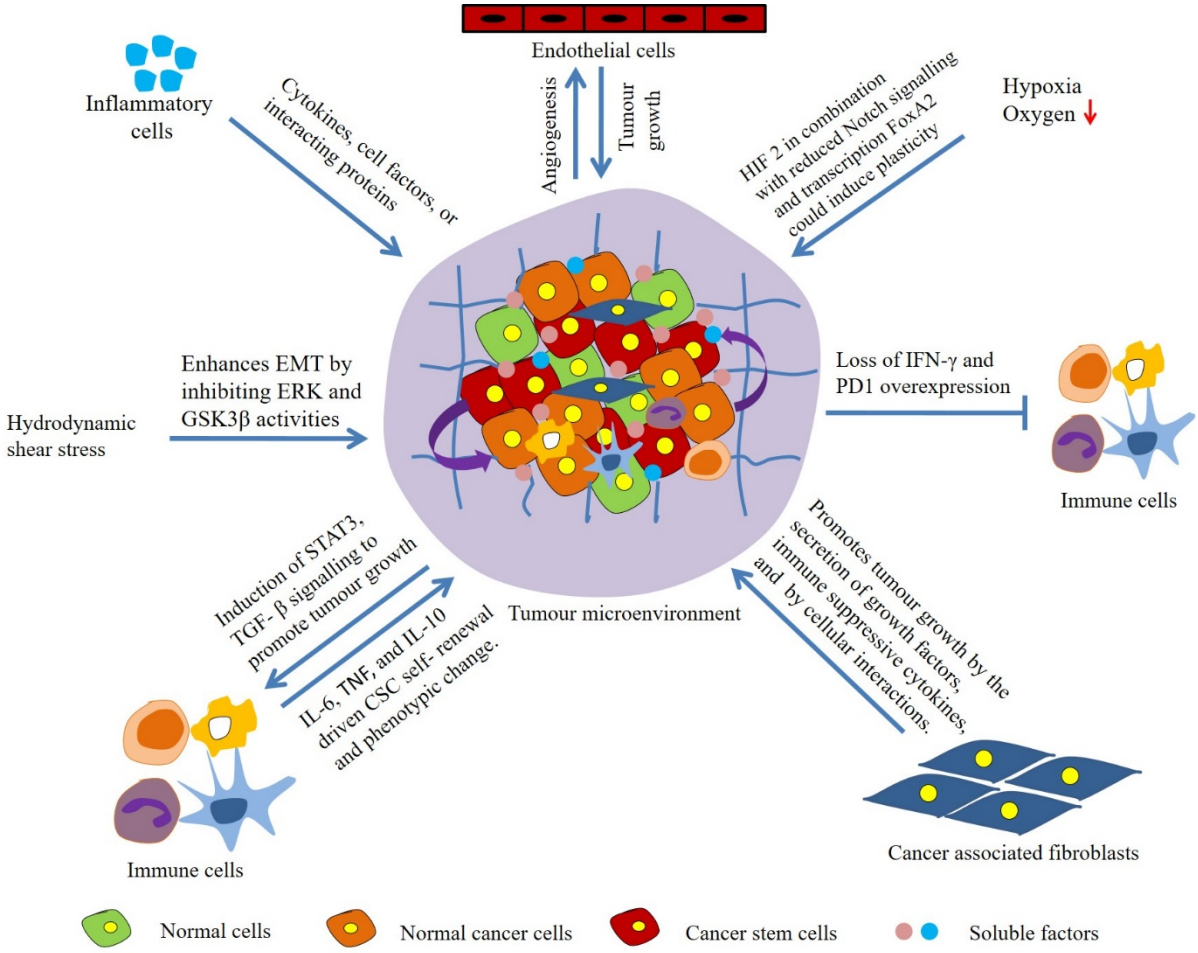

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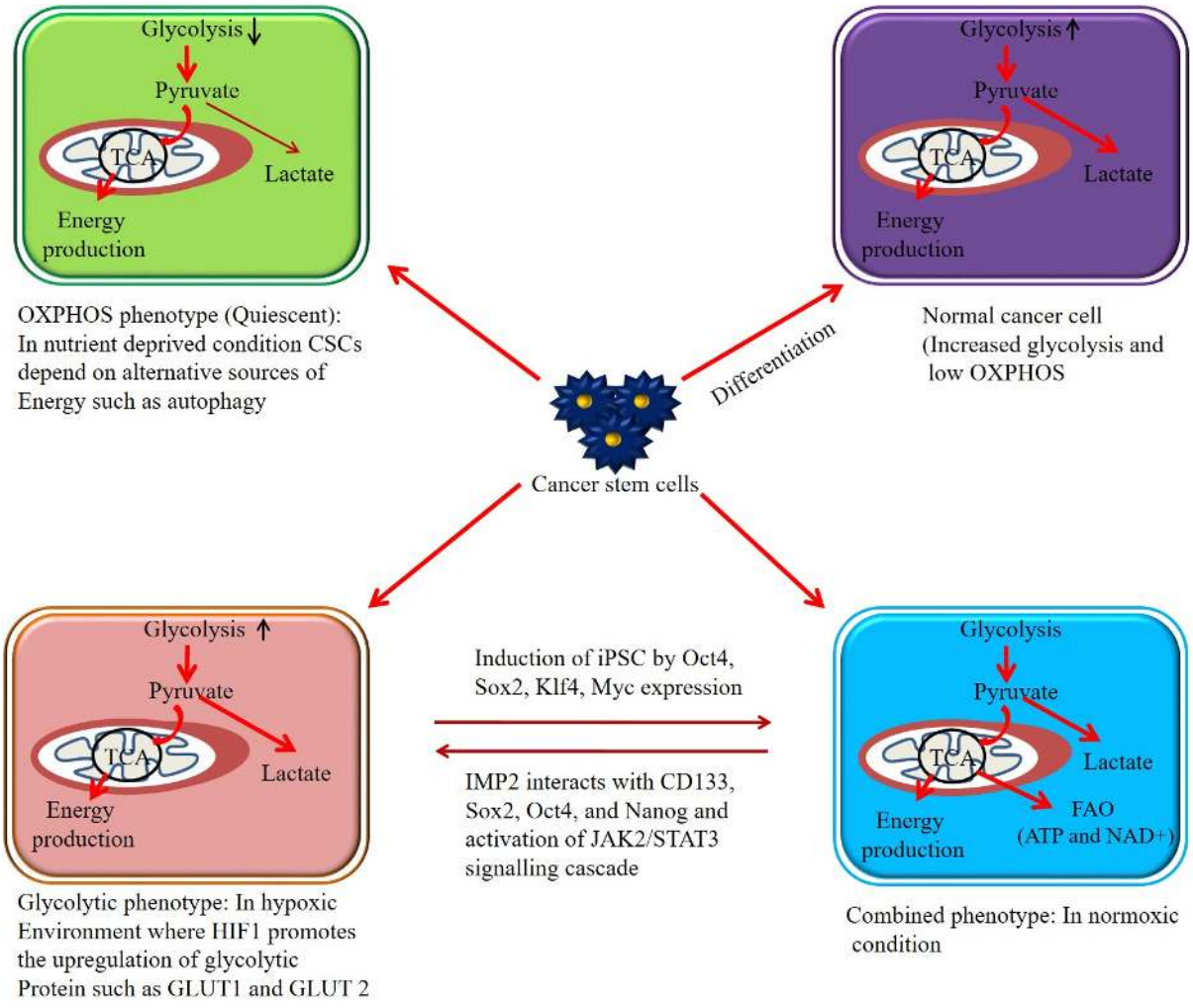

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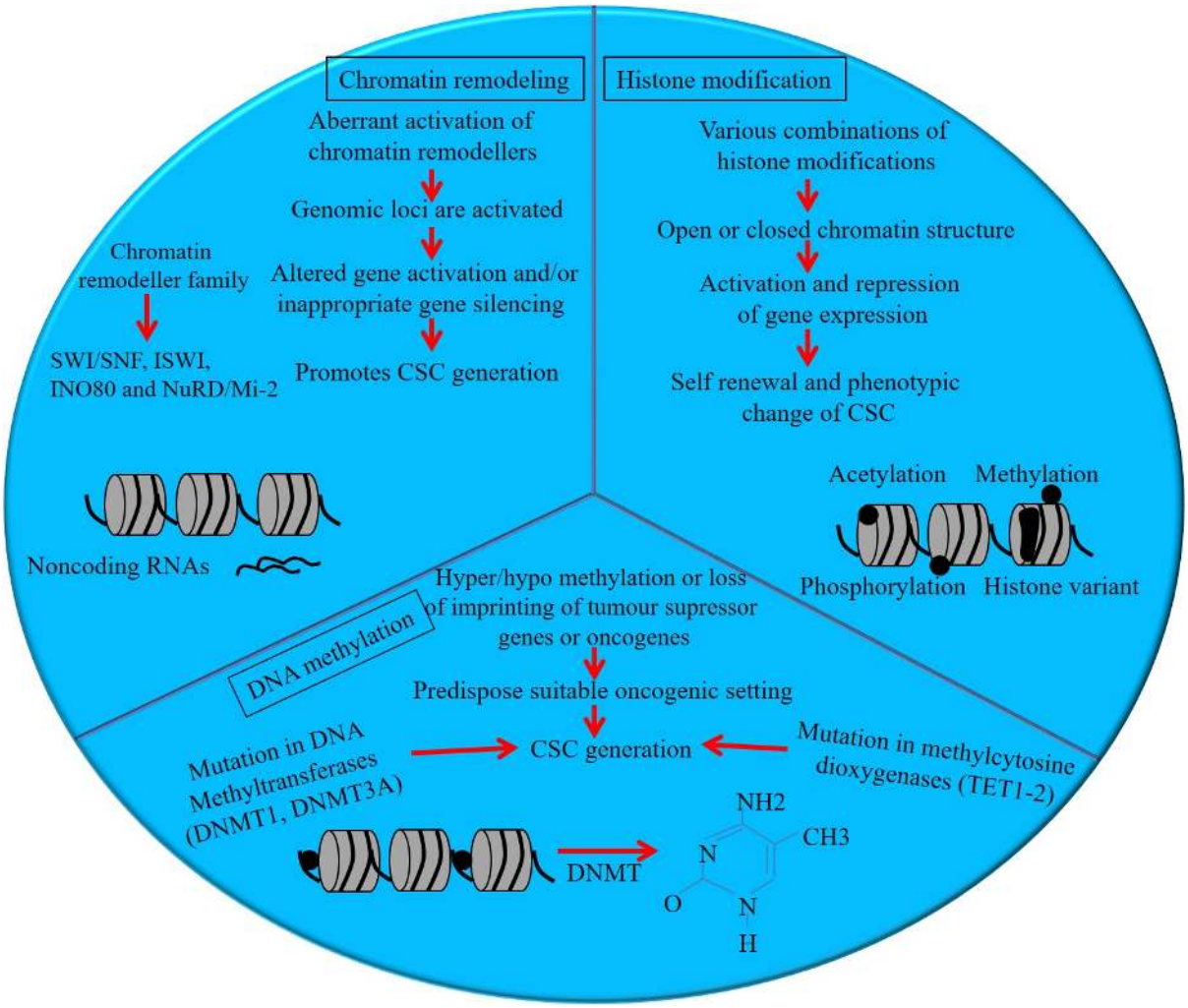

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