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The Enemy Within: Influence of Cancer Stem Cells in Neoplastic Progression, Metastasis, Immune Evasion and Resistance to Therapy

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Cancer stem cells are the prime malefactors of tumours, which create all the havoc in patients. Cancer originates with enormous heterogeneity and exists in a massively complex environment. In that complex environment, Tumour makes its way to success using all forms of capabilities to cope and defeat the defence system of the body as well as clinical approaches. Cancer stem cells act as the Trojan horse in this bodily war. For last one decade researchers are trying to generate a clear map about Cancer stem cell niche generation, regulation of quiescence and therapeutic response. In this study, we have reviewed the advances on different stem cell models, isolation strategies, characteristics, contribution towards cancer development and therapeutic targeting using a combination of chemotherapeutic agents with nanoparticles.

Key words: CSC, Tumor microenvironment, TAM, Hypoxia, HIF-1α, TNF-α, PGE2

Abbreviations: AML-Acute myeloid leukaemia, CSC-Cancer stem cells, MDSC-Myeloid-derived suppressor cell, CTC-Circulating tumour cell, TAM-Tumour TME-Tumour Microenvironment associated Macrophage, TNF-Tumour necrosis factors.

1. Introduction

Since the discovery of antibiotics, infectious disease related mortality has gone down globally while neoplastic diseases became leading cause of mortality and emerged as a road block for farther improvement of life expectancy worldwide. It is estimated globally by International Agency for Research on Cancer that 1 in 5 people develop cancer in their lifetime, of which 1 in 11 women and 1 in 8 men die from this disease. Globocon 2020 reported that the global cancer burden has risen in the number of total cancer cases as 19.3 million and 10 million death [1].

In modern world, many strategies for cancer treatment have been developed including radiotherapy, chemotherapy and surgery. But the main reason for cancer prevalence is metastasis, cancer recurrence, evading immune surveillance, resistance to chemotherapy and radiotherapy and heterogeneity. In advanced cancer, cellular heterogeneity serves as an important cancer hall hallmark, which plays the most crucial role in cancer aggressiveness and therapeutic resistance. Cancer stem cell is an important asset of tumour niche, which possess the top rank in the hierarchy of cell populating and regulating tumour microenvironment, with capability to generate differentiated cancer cells. Also, contribute to therapy resistance, recurrence and tumour niche maintenance. So,for successful tumour growth inhibition Cancer stem cells must be dealt with.

The origin of the term "stem cell" traces back to 1868, drawing of phylogenetic trees by eminent German scientist Ernst Haeckel, representation of evolution and named these trees "stammbaume" (German for "stem trees"). In 1877 he describes the unicellular fertilised egg cell as "Stammzelle" which can propagate all other cells of a multicellular organism. At around 1900 Stem cells get a place in embryological studies of Theodor Boveri, August Weishmann's theory of 'germ plasm', Ernst Neumann Artur Pappenheim's investigation on haematopoiesis and leukeamia.The origin of Cancer stem cells is a hotly debated issue, R Virchow proposed embryo-like cells having potential malignant capabilities reside in the body at dormant stage from birth [2]. A student of Virchow, Julius Cohnheim proposed tumour develop from rudiments or displaced embryonic cells, theory develop as 'embroyonal rest theory'. From his definition tumour is 'atypical neoplasms of tissue based on an embryonic rudi-

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ment'. In his view malignancy of tumour depends on lack of 'physiological resistances' [3]. In the medicinal field 'Cohnheim's theory' get wide attention in the early twentieth century. And tumour arises as a mixture of differentiated and undifferentiated tissues. The modern view of cancer stem cells is not completely different from them. The term "cancer stem cell was introduced by Reya, Morrison et. al. in 2001.

Cancer stem cells or CSCs have been found in a variety of tumour types, including acute myeloid leukaemia, pancreatic cancer, lung cancer, hepatocellular carcinoma, breast cancer, head and neck cancer, melanoma, prostate cancer, and glioblastoma[4]. Studies revealed that cancer stem cell suppression in clinical trials has resulted in tumour eradication, cancer recurrence and resistance to chemotherapy and radiation therapy increases with CSCs no[5]. Stemness of these cells is maintained by tumour microenvironmental stimuli collectively modifying self-renewal pathways such as Hedgehog, Want/B-catenin, and Notch pathways, or by regulating master transcription regulators such as NF-kB, K-Ras. Nonetheless, actual regulating mechanism controlling dynamic CSCs features is still not much understood.

2. Cancer stem cell Model

From its very first origination tumour is known for its heterogeneity. Tumour consists diverse types of cells with different molecular signatures. Two major CSCs model of tumour heterogeneity was illustrated over the decades. One is the hierarchic model and second is the Stochastic model.

Hierarchic model/cancer stem cell model: Hierarchic model elucidate that precursor stem cell give rise to cancer. Precursor cells with high clonogenic properties symmetrically or asymmetrically give rise to two identical CSCs or only one cell [6]. Hierarchic model supports the ability of a self-sufficient CSCs population, who resides at the top of the hierarchy to give rise heterogenous cancer cells. Which are capable of self-renew as a result sustain stem cell pool. John Dick and colleague study on AML supports that stem cell phenotype containing population maintained throughout the tumour development in TME with tumorigenic capability [7]. These CSCs are the source population of tumour development and heterogeneity. This model suggests CSCs specific properties can be used for detection and isolation of CSCs, also for developing specific CSCs targeting therapy [8].

Origin of CSCs, and their cell surface markers are an intensely debated field in the field of CSCs model. Now a day various recent studies in some human cancer are supporting this model as malignant glioma, adenomas. Saying mostly differentiated cancer cells originate with limited cancer forming properties derived from cancer cell subpopulation having stem-like features are the major player of tumour maintenance [9] [10].

A few as a hundred of breast CSCs who reside at the top of the hierarchy reported being able to develop breast tumour in mice [11]. This model suggests origin of intra-tumoral heterogeneity differently, but this models are not mutually exclusive. Because in TME major genetic events instigate CSCs and their progenies to give rise to new clones with new genetic complexities. New cloned cells may or may not fit in hierarchical structure. Results of some observation of hierarchical model reported cancer recurrence after successful eradication of CSCs [12].

Stochastic model: According to stochastic model all tumour cells are equally potent to form tumour. They can accumulate epigenetic change and may promote tumour aggressiveness, invasiveness and therapy resistance [13]. The stochastic model explains a clonal evolution influenced by intrinsic factors derived from cancer. This model suggests that every cancer cell can self-renew and can acquire disease characteristics because of the ability to interconversion of cell phenotypes [14].

Tumour growth resulted from accumulating somatic mutation acquired by genetically unstable cells. This model says each cancer needs specific therapy because of random mutation. This report supported by differentiated cells after eradication of CSCs can switch to stem-like progenitors and promote tumour development [15]. This model does not address properly about phenotypic variations of CSCs in different clones.

3. Origin of Cancer Stem Cells

The existence of CSCs reported from various cancer including AML Acute myeloid leukaemia, Hepatocellular carcinoma, breast cancer, melanoma, lung cancer, glioblastoma and many more [16]. Signalling pathways which are related to normal stem cell physiological functioning are dysregulated in CSCs like JAK/STAT, NF-kB, c-MYC, HER-2, Hedgehog, PTEN/PI3K, etc [17]. Over expression of normal stem cell markers like Nanog, Sox2, Oct 4 has enlisted. However, the origins of CSCs are not fully understood. Kleinsmith and Pierce demonstrated the ability of a single embryonal carcinoma cell to form a malignant tu-

mour in 1964 [18]. A random mutation in a normal cell during DNA replication can transform it into a CSC. Activation of constitutive Wnt signalling, which is common in normal stem cell affects the self-renewal of mammary stem cells resulting metaplasia and adenocarcinoma [19].

The main mechanisms which give rise to CSCs are genomic instability, microenvironmental changes and gene transfer **(Fig. 1)**. In stem, progenitor, and differentiated cells, genomic instability is the fundamental basis of cell transformation, which leads to cancer initiation [20]. With having unlimited growth capacity, it is thought that stem cell transformation requires only a few genomic changes. More than 10% of gastric cancer identifies with low mutagenic change, which suggests this cancer originate from stem cell.

Fig. 1. Mechanisms which contributes in CSCs transformation.

A variety of microenvironmental factors can influence transformation and CSC differentiation. Various inflammatory cytokines, tissue injury, toxin exposure, radiation treatment can cause mutations in tumour suppressor genes or oncogenes. Stem cells possess CSCs derived IL6, which aids in the differentiation of non-CSCs into CSCs. In a study normal cell transformation to malignant cells by 40 weeks of cadmium exposure has been also reported. Mice were injected cadmium-treated cells, which resulted in invasive, metastatic carcinoma [21].

Horizontal gene transfer is common in normal and cancer cells causes origin of CSCs [22]. Tumour cells may take up fragmented DNA by phagocytosis, resulting CSCs formation by genetic alteration [23].

Fusion of tumour cell with other cells can give rise tumour stem cells. Various data supported this cell fusion concept. Tumour cells fusion with lymphocyte can result diversification of tumour cell. Stem cell fusion with mature cell and resulting multinucleated CSCs like cell formation has been reported by Pomerantz and Blau et al. Fused cell isolated from tumour have more invasive and migration capability. Cancer stem cell and cancer cell-derived factors such as various Proteins, chemokines, cytokines as IL4, CD44, CD47 causes cell fusion [24].

Metabolic reprogramming may cause cancer stem cell dedifferentiation from non-CSCs. Hypoxia induced breast CSCs population increased has been reported in 2012. Switch from oxidative phosphorylation to glycolysis in somatic differentiated cells resulting somatic cells reprogramming into pluripotent cells [25].

Having immense heterogeneity origination of CSCs is a difficult task to trace.

4. Isolation of CSCs

CSCs comprise a very small proportion of tumour generally 0.01–2 percent only. Additionally, CSCs possess characteristics of normal stem cells, So the identification and isolation of CSCs from a huge mass of non-cancer cells is a challenging issue. In recent times various strategies adopted by researchers to isolate and detect CSCs. Proper understanding of CSCs hierarchy is still not clear, so for isolation generally stem cell markers and stem cells specific transcriptional molecules are used to identify CSCs. Very recent studies where utilization of transplantation assay was used to establish the presence of CSCs [26].

Sphere colonies: Sphere formation capability of cancer stem cells in culture in serum free media containing basic growth factors are used for Cancer stem cells. In this media immature cell grows and form floating spheres, non-cancer cell or differentiated cell die out [27]. Isolated cancer stem cells from cancer patient shows spheroid formation capacity. But this method has very low efficiency consist many drawbacks.

Side population: Cancer stem cell shows high drug resistance by efflux out drugs using ABCG family of membrane transporters. They express high amount of ABC transporters, which contribute towards CSCs chemotherapeutic efflux [28]. By measuring fluorescent dye efflux capabil-

ities, CSCs can be isolated. These low-fluorescent expressing side population or negatively stain population isolated and inoculated to mice shows successful cancer development in immune compromised mice [29].

But as dye has toxic effect on cells and for having low purity and specificity of cells this method is not very efficient in isolation of CSCs.

Cellular markers used for identification of Cancer stem cells: Biomarkers located on the cell surface are primarily used for CSCs isolation. FACS based cell sorting techniques are now a days most effective and widely used technique for CSCs separation.

Table 1: Containing Various Markers used in Studies for Identification of Cancer Stem Cells in Different Cancer Types

Cancer type	Surface marker	Intracellular Marker and Signaling Pathway	Reference
Lung cancer	CD44, CD90, CD87, CD133, CD166, EPCAM	ALDH, Nanog, Oct3/4	[32] [33] [34] [35] [36]
Breast cancer	CD24, CD29, CD44, CD491, CD61, CD70, CD133 CXCR4, EPCAM, LGRS	ALDH, Bmt-1, Nanog, Notch, SOX2, Oct3/4	[37] [38] [39] [40]
Gastric cancer	CD24, CD44, CD90, CD133, CXCR4, EPCAM, LGR5, LINGO2	ALDH, Let m1, musashi 2, Nanog, Oct3/4, SOX2	[41] [42] [43] [44]
Liver cancer	CD24, CD44, CD90, CD133, EPCAM	AFP, Nanog, Notch, Oct3/4, SOX-2, Wnt/catenin	[45] [46]
Colo-rectal cancer	CD24, CD44, CD133, CD166, EPCAM, LGR5	ALDH, Let m, Nanog, Oct3/4, Sa114, SOX2	[47] [48] [49]
AML	CD33, CD123, CLL-1, ITM3	ALDH, Nanog, Oct3/4, SOX2	[50] [51]
CML	CD25, CD26, CD33, CD36, CD117, CD123, IL1RAP	JAK/STAT, Wnt/Beta-cat, FOXO, Hedgehog/ Smo/Gli2	[52] [53] [54]

Tumour microenvironment contains connective tissue, components of extracellular matrix and a huge number of infiltrating cells including various Immune cells, endothelial cells, stromal cells and provide signals to Cancer stem cells and this interplay helps Cancer stem cell to maintain dormant state for years and causes tumour recurrence. Since then several studies have been reported for identification of specific markers for CSCs, but a standard marker for CSCs not yet been discovered. Markers for isolating stem cells are proteins or glycoproteins various CD markers and signalling pathways. CSCs were isolated from leukaemia for the first time in the 1990s using the surface markers CD34+ and CD38−. Isolated cells were found to be similar to normal hematopoietic stem cells and capable of initiating Acute myeloid leukaemia in NOD/SCID mice [30]. Genetic modification such as elevated ATP-binding cassette, Multi drug resistance phenotype, mutated NOTCH1 expression, ALDH high in CD44+/EPCAM+ cells as superficial markers for NSCLC stem cells are reported to use in CSCs studies [31].

CSCs are isolated by expression status of various CD markers such as CD34, CD44, CD47, CD24, CD90, CD133 and many more (**Table 1**) as well as proteins or glycoproteins and signalling pathways.

4.1 Surface Markers Used for Isolation of Cancer Stem Cell in Various Studies

Other isolation strategies: CSCs escape chemotherapeutic drugs because they are mostly static and remain in Go phage. This property of drug resistance can be utilised for CSCs isolation [55]. Percoll density gradient centrifugation was used in a study for hepatoma stem cell isolation [56].

5. Cancer Stem Cell and Tumour Microenvironment TME

Tumour micro environment/TME is a complex environment consisting of huge no. of cells and factors with low oxygen and low nutrition available (**Fig. 2**). Effect of tumour microenvironment on CSCs is still in a very elusive state. Stem cells resides in a niche-specific microenvironment in both normal as well as in cancer condition. CSCs makes this stem cell specific niche by interacting TME components for supporting its well-being in the microenvironment. By establishing this complex interaction of CSCs on its niche building and management will have a greater impact on tumour therapy. Majority of tumor tissue support heterogeneity, a mixture of self-replicating and non-replicative tumour cells [57].

CSCs by activating stem cell self-renewal and regulating other tumour comprising molecules behaviour dictates tumour micro environment. CSCs interact with TME using a wide variety of soluble factors, micro vesicles and exosomes. IL-6, IL-8, IL-1beta, VEGF, HIF-1alpha, MMPs, CCL5, or CCL2 can be secreted via exosomes and other infiltrating cells [58]. They promote development of the aggressive tumour promoting TME. Many of these signalling molecules and tumour infiltrating cells, stromal cells and CSCs support a communication loop which regulate TME. TME possesses both acidic and hypoxic

environment. Which can modulate behaviour of cells by metabolic reconfiguration, cell migration, cells commitment to environment, Epithelial to mesenchymal transition, metastasis, angiogenesis also regulating self-renewal and potency associated factors as and intracellular signalling pathway as Nuclear factor-kB, Hedgehog, JAK-STAT and TGF/SMAD, PI3K/AKT/mTOR and transcription factors as Nanog, KLF4, OCT4 and MYC Snail, Twist [59].

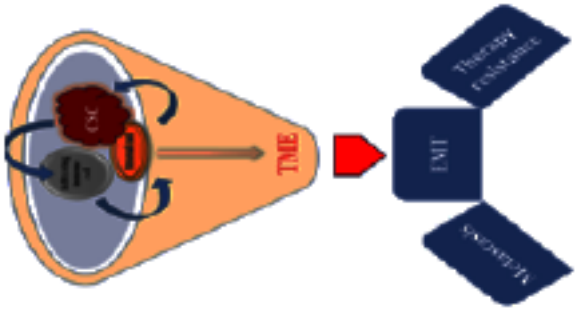

Fig. 2. Infiltrating immune cells, CSCs, Stromal cells participate in maintenance of TME and lead tumour progression.
TME-Tumour micro environment, CSC-Cancer stem cell, EMT-Epithelial to mesenchymal transition

There are some key players in TME who play vital roles in the building up and maintenance of TME and influence profoundly to the CSCs and TMEs to acquire their perspective fate. These are Hypoxia, Tumour infiltrating immune cells, Stromal cell constituents of TME, Adipocytes, etc.

Hypoxia: Sufficient Oxygen is essential for normal development of any cell, but oxygen supply decreases with developing tumour stage. Low oxygen conditions called hypoxia which has been found to create an immense impact on cell development and metabolism. Hypoxia start to change cells metabolism and fate of development. Hypoxia was reported to help embryonic fate development of cells [60]. Unfortunately, as we knew cancer knows the best about how to use normal cellular functioning for their well-being, so hypoxia is not an exception. HIF-1 alpha, HIF-2 alpha in neuroblastomas were reported to decrease several neuroendocrine markers but increases neural crest progenitor specific Notch-1 c-Kit expression [61]. Another study of neuroblastoma cells reported that hypoxia mediated increased ID2 associated with blocking dedifferentiation of cells and promote fundamental traits of cancer as angiogenesis, tissue invasion and increased proliferation [62]. Various detailed report of hypoxia mediated EMT progression have also been enlisted as Snail, twist and HIF-1 alpha expression on HNSC help in metastasis and EMT [63]. Prime stemness inductive signalling pathways as WNT/b catenin, Notch, Hedgehog all are associated with hypoxia resulting EMT progression [64].

These previous reports suggest that hypoxic TME can have a major impact on CSCs which have metastatic properties.

Immune cells: The interplay between tumour infiltrating immune cells and CSCs in the TME is a topic of greatest importance among researchers. After all, TME is an area of chronic inflammation which shapes tumour development [65].

Huge infiltration of immune cells in the TMEs and their pro tumorigenesis strategies are well known. Communication of these immune cells with CSCs mutually helps in the maintenance of immune suppressive TME. Immune suppressive nature of TME is the main reason of tumour uncontrolled growth and spreading. Various research establishes that CSCs is a major player for the building up and maintenance of immune suppressive TME. CSCs reported to express CD200, Fas-L, Bcl-2, CD47, NK cell inhibitory receptor and ligands, HLA-I and reduced MHC molecule on its surface [66]. CSCs remodel immune system toward immune suppressive characteristics by promoting MDSC recruitment, M2 polarisation, T regulatory cell recruitments and by inhibiting effector immune cell like macrophage, T cells, NK cells and DC cells [67] [68]. CSCs regulate the recruitment and activation of various immune cells such as macrophage, T regulatory cells, Lymphocytes, Myeloid derived suppressive cells. Secretion of pro-inflammatory cytokines and chemokines from CSCs such as IL-6, IL-4, CCL2, TGF-beta mediated recruitment of macrophages reported in various types of cancer [69] [70]. CSCs, not non-CSCs are found to be in a different metabolic state influenced by TMEs. Breast CSCs are shown to be more dependent on oxidative phosphorylation whereas non-CSCs with more aerobic glycolysis [71].

CSCs influence the ability of innate and adaptive prospects of immune system to detect and eliminate cancer cells. This aspect was detailed reviewed by Vesely and Kershaw et.al. [72]. Low immunogenic properties of cancer stem cells and its ability to cycle between quiescent stages, keep them safe from immune recognition and elimination [73]. TAM and CD4+ T cell mediated TNF alpha secretion resulting to induction Snail, Slug, Twist mediated self-renewal of CSCs [74].

CSCs derived TGF-beta were reported to induce naive CD4 + CD25− T lymphocytes differentiation to CD4+CD25+, FOXP3 expressing T regulatory cells and help in the maintenance of immune suppressive nature of TME [75]. Treg cells regulate pro tumour immunity by regulating T lymphocyte accumulation to TME and also by secreting TGF-beta and IL-10 which in turn help CSCs, and promote tumour survival and growth.

Stromal cell: Stromal cell constitutes a huge portion of tumour microenvironment cells. Stromal cells influenced by TME start to develop tumour promoting properties. Stromal cells were found to be more genetically stable rather than tumour cell. So, these cells could be an attractive therapeutic target to reduce tumour prevalence. Stromal cell as mesenchymal stem cells, carcinoma associated fibroblast, adipocytes start to produce a wide variety of growth factors as PDGF, VEGF, HGF and cytokines which interact with CSCs and cooperatively maintain TME [76].

Carcinoma associated fibroblast and Cancer stem cells are two important co-conspirators of TME, which mutually support tumour growth. Fibroblasts are very abundant cell type of TME (up 80% of the tumour mass in pancreatic tumours) and their crosstalk with CSCs and infiltrating immune cells leaves a huge impact on cancer development [77].

Normally fibroblast upon activation differentiated to myofibroblast and helps in wound healing and fibrosis, in TME differentiate in to CAF, carcinoma associated fibroblasts. CSCs and CAF coculture reveal that through paracrine network CAFs enrich CSCs by reacquisition of stem cells like properties. Various growth factors life VEGF, TGF-beta, PDGF and various ECM components like MMPs, collagen, fibronectin that support TME maintenance and tumour spread [78]. In presence of CAF expressing IGF-II, IGF1R signalling activation in cancer cell induce Nanog expression and stemness increasement [79]. Cancer associated fibroblast can regulate cancer stem cell function, in mammary gland tumours CSCs mediated Hedgehog ligand SHH expression regulate CAF via Hedgehog signalling activation. CAF secreted factors regulate cancer stem cell self-renewal and expansion. Hedgehog inhibitors reduce tumour growth by inhibiting CAF and CSCs expansion [80]. In TME CAFs populations is the main source of multifunctional cytokines such as IL-6, IL-8 which regulate the balance between CSCs and differentiated cells [81] [82].

Newly isolated CAF population CD10+/GPR77+ with active NF-kB signalling release IL-6 which correlated with stemness induction and poor survival of patient in lung and Breast cancer [83].

It is a well-known fact that Fibroblast is the principal regulator of the process of wound healing. Cancer emerges with the tagline "the wound that does not heal" prevalence of the wound caused by progenitor and stem like cells and other co-operators who support stemness [84]. So, focusing on CSCs-CAF partnership is an important aspect to understand their control towards each other and to the TME.

Adipose tissue constitutes a huge portion of TME which contains adipocytes and various other stromal cells. Besides lipid storage function adipocyte also secrets several types of cytokines and adipokines such as IL-6, TNF-alpha, MCP-1, leptin and adiponectin [85]. These cytokines are reported to involve in immunomodulatory functions like recruitment and activation immune cells specifically macrophages. These cytokines also regulate CSCs directly. Leptin is reported to be upregulated severely in CSCs. Leptin activates STAT3 in CSCs enhance OCT4, SOX2 expression which in turn stimulate leptin receptor on adipocytes [86]. Coculture of adipocytes with CSCs reported to increase cancer stemness [87]. Colorectal cancer derived adipocytes were found to promote cancer cell stemness [88].

5.1 CSCs Influence Towards Circulating Tumour Cell

In cancer field, wide attention has been attracted by circulating cancer cells and cancer stem cells. These two cells have major impact on cancer recurrence and therapy resistance and fatalities regarding cancer. Various studies reported that small subset of CTCs contains stem like features and is capable to undergo EMT called circulating cancer stem cell [89] [90].

In breast cancer, colorectal cancer, Prostrate cancer CTCs number in metastatic patients act as a predictor of overall survival of patient [91] [92] [93]. Circulation tumour cell origination and their contribution toward metastasis remain elusive. But presence of a small population of CSCs with CTCs indicate that, CSCs which after entering to circulation get identification as CTCs. CTCs isolated from melanomas have been reported to have metastasis forming capabilities [94].

Further insight in this aspect will lead to understanding more about the contribution of CSCs in metastasis development. CTCs and CSCs both overexpressed stemness and EMT promot-

ing genes [95]. In CTCs population presence of 35.2% CD44+/CD24− and 17.7% ALDH1 high cells demonstrated in 66.7% of breast cancer patient. So distinguishing CTCs from the population CSCs is a challenging aspect. As CTCs reported a poor prognosis of cancer so the extensive study is needed to elucidate the role of CSCs control toward CTCs for developing a successful therapeutic approach.

Metastasis is a multistep process involving spreading of tumour to the secondary tumour site in which some tumour cell migrates from its primary site to distant organ, where they initiate colonization and secondary tumour formation. The migration of cells is critical in normal developmental process. For successful migration epithelial mesenchymal transition (EMT) is a key program. Some adult tissue maintains migration capacity by generating epithelial mesenchymal transition at the time of wound healing, tissue regeneration and remodelling. The process of EMT induction is maintained by expression of CSCs markers, enhanced self-renewal correlated with tumour growth. A direct relation between EMT and gain of epithelial stem like properties was reported in mouse and human mammary carcinoma [96].

5.2 Cancer Stem Cell Influence in Prognosis and Treatment

Growing body of research supports tumour microenvironment associates and help cancer stem cell to adopt drug exposure. Adult stem cell niche maintains its stemness as well as inhibit tumorigenesis by using inhibitory signals to proliferation as well as differentiation. When chemo or radio therapeutic drugs were administered to TME than only differentiated population get eliminated but the undifferentiated or self-renewable CSCs subsets escape (**Fig. 3**). They can initiate tumour and continue to grow. For acquiring proper therapeutic success in clinical ground, elimination of this stem progenitors is necessary.

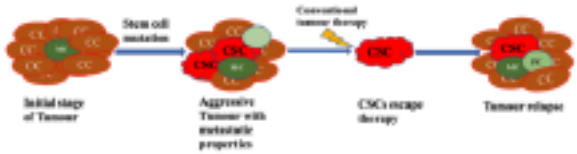

Fig. 3. CSCs escape conventional therapy and causes tumour recurrence. CC: Cancer cell, MC: Mature cell, CSC: Cancer stem cell, PC: Progenitor cell

CSCs distinguish themselves by their ability to remain a slow growing phase of dormancy. This quiescence nature preserves them from conventional tumour therapies. Research support that CSCs marker specific cells survived neoadjuvant chemotherapy treatments [97]. Despite several research approach in the past CSCs influence to cancer therapy is still very elusive. Influence of cancer stem cell on therapy resistance can be either intrinsic or acquired. Intrinsic factor present on TME can interact to CSCs and push tumour development and therapy resistance by helping one another. Or by Acquired drug resistance which develops after cancer therapy. CSCs which are therapy resistance carry out tumour metastasis by pushing and guiding development of premetastatic niche and supports 'seed and soil' hypothesis [98]. Cancer acquire drug resistance after treatment with the help of CSCs population having ability to expel out drugs from cells [99]. One of the famous drug resistance mechanism of CSCs is by using transport proteins ATP binding cassette family. These proteins effectively efflux drug out of the CSCs. CSCs reported to overexpressed these transporter proteins [100] [101]. Several chemotherapeutic drugs resistance is reported to correlated with ALDH expression in CSCs [102].

ALDH catalyses aldophosphamide to carboxyphosphamide oxidation method primarily used by normal cell for cyclophosphamide detoxification. CSCs hijacked this mechanism to deal with cyclophosphamide and some chemotherapeutic drugs [124]. CD133+ CSCs from Glioma express high MDR-1 and Bcl-1 [103].

CSCs make themselves resistance to DNA-damage mediated cell death by increasing ROS scavenging, ATM and CHK1/CHK2 mediated repair promotion, activating anti apoptosis signalling [104] [105]. Autophagy is a physiological process which supports generation of intracellular nutrient on the basis of needs. CSCs reported with increased autophagy in various cancer types [106]. Autophagy inhibition in CSCs could lead to reduce chemoresistance [107].

6. CSC and Radio Resistance

Radiotherapy is one of the most feasible approach adopted for cancer treatment. CSCs are reported to be more radioresistant. Pro survivalability of CSCs reported to protect these cells and help to adopt radiation resistance. CSCs adopt different strategy to develop radioresistant. CSCs enrichment increases following radiation, this can be the result of differentiated cell killing out from tumour and the ability of CSCs to escape radiation. Following RT neoplastic cells heterogeneity

help clonal evolution and lead to develop adaptive response to radiation and promote tumour aggressiveness [108]. Radioresistant enhancement is related with increased self-renewal and reduced DNA damage by decreasing ROS species. Enrichment supported by efficient DNA damage repair system of CSCs.

DNA double stranded break repair is shown to be higher in CSCs than non-CSCs, homologous recombination repair in MDA-MB231 derived stem cell phenotype containing cells reported with more radioresistant [109]. Radiation resistance in MCF-7 and MDA-MB231 derived CD44+/CD24 low cancer stem cell markers specific cells targeting using ATM inhibitors found to decrease [110].

ROS is an important factor of normal cellular physiology, such as differentiation, metabolism, proliferation and apoptosis etc. Intracellular ROS scavenger upregulation is reported by multiple studies in CSCs. Low ROS reported with increasing radioresistant acquired by CSCs [111] [112].

Normal stem cell development pathways which are reported to regulate self-renewal also related to radiation resistance of CSCs [113]. Stem cell developing key regulatory pathways also help cancer cell to acquire radioresistant by switching self-renew.

Mouse mammary CSCs are reported to increase radioresistant depending on WNT/beta-catenin pathway [114]. Wang et al. demonstrated in Glioma model Gamma-secretase inhibitor targeting to NOTCH pathway increases CSCs sensitivity toward radiation [115]. Oct-4 expression in CD133+ cells in lung cancer correlated with radioresistant. Knockdown of Oct4 reported to improve treatment efficacy [116]. These reports suggested that self-renewal ability acquisition is one of the most important strategy of CSCs to develop resistance to radiotherapy. In lung cancer CD133+ cells with altered DNA repair system which can induced by chemotherapy resulting DSB resolution and accumulate radio resistance. Ionizing radiation resistance is involved with the upregulation of DSB genes such as BRCA1, Rad5, Eso1 [117].

Evidence of radio resistance mediated by CSCs is increasing and the plausible understanding of this mechanism will help to improve cancer therapy in near future. Clinical reports about this aspect is still scarce. Issues like identification and difference from healthy cells and plasticity are still in its infancy.

7. Cancer Stem Cell and Chemoresistance

Recent day Chemotherapy is an important mainstream anticancer targeting strategy. Chemicals having the capability to inhibit mitotic division, induce DNA damage or apoptosis are used for anticancer therapy for decades [118].

Chemotherapy resistance is acquired by slowly dividing cells and non-dividing cells. Knowledge about how tumour became chemo resistant is still growing day by day. CSCs emerges as a crucial player in chemoresistance development. Variety of chemotherapeutic agents as temozolomide, paclitaxel, etoposide, doxorubicin, cisplatin treatment reported in various types of cancer as glioblastoma, breast, colorectal, lung, bone, ovarian and prostate cancers reported CSCs mediated therapy resistance development [119].

A series of self-defense mechanisms adopted by cancer stem cells to cope with chemotherapeutic agents. Deregulation of various developmental pathways are responsible for drug resistance in CSCs. Hedgehog, Wnt/beta-catenin, Notch pathways are reported to influence chemoresistance in CSCs. CD44+/CD24-breast cancer stem cell isolated from xenograft model with high Hedgehog signalling. Temozolomide treatment resistance mediated by Notch and Hedgehog pathway in CD133+ CSCs reported from Glioma model [120]. Detailed review of WNT/beta catenin signalling and its control to chemoresistance is available [121]. Inhibition of WNT signalling found to decrease CD44, ALDH1 expression status and reduced tumour sphere formation in Breast cancer[122].

Induction of stemness properties on CSCs mediated by Hippo pathways via activation of YAP/TAZ also reported in Breast cancer model [123]. CSCs reported with poor expression of death receptors. AML derived CD34+ CD38− CSCs express poor level of FAS and FAS-L, promoted chemoresistance. Glioma model targeted with synthetic FAS-L and temozolomide reported promoting apoptosis of stem phenotyping cells [124] [125]. Bortezomib a chemotherapeutic agent in combination with Soluble recombinant Trail found to reduce colony forming capacity in glioblastoma derived CSCs [126]. Apoptosis inhibitor protein such as XIAP that confers resistance against γ-irradiation was found to be overexpressed in Glioblastoma initiating CD133+ GBM cell compared to non-CD133+ counterparts [127].

Expression of Pro and anti-apoptotic gene fam-

ilies are reported to be dysregulated in CSCs which help them to escape apoptosis in response to chemotherapeutic drugs [128]. Breast cancer stem cell resistance targeting with paclitaxel and doxorubicin is related with ABCB1 over expression mediated drug efflux. Multiple myeloma cells treated with carfilzomib confers ABCB1 mediated resistance.

8. Targeting CSC with Chemotherapy and Nano-medicine

As cancer stem cells are the prima facia of cancer recurrence, eradication of cancer stem cell came out as the most needful strategy to improve cancer therapy. Researchers for many years are trying to develop many strategies towards CSCs. Among them chemotherapy, radiotherapy, immunotherapy is mostly used. Conventional drug fails to cure cancer because of so many reasons, like drug paclitaxel which target proliferating cancer cells but CSCs got escaped because of its dormant nature. These dormant, multidrug resistance cells cause tumour relapse [129].

Normal proliferative cells also become victims of chemotherapeutics drugs. As example, cyclophosphamide has been seen to kill both dormant and proliferating cells [130]. CSCs targeting by using kinase inhibitors or using prime signalling pathways such as Notch, Wnt, Beta-catenin inhibition is already entered the clinical world. The most well-known and well-established tumour treatment is using chemotherapeutics. But with growing tumour CSCs acquired chemo resistance characteristics which eventually leads to failure of tumour therapy and recurrence of cancer [131]. Conventional chemo and radiotherapy were reported to reduce tumour burden initially but after a few days recurrence and relapse of cancer by drug resistance CSCs is an obvious event. In conventional therapy normal tissue also gets harmed by many side effects as neuropathy [132], bone marrow suppression [133], alopecia [134] etc.

Therefore for enhancing efficacy and for reduction of side effects design of a drug delivery system to target CSCs emerges as the best solution. That is why Nanomedicine strategy comes in the field of cancer therapy. Nanosized drug delivery system reported to increased half-life and bioavailability of the drug. Only a handful of high efficacy drugs are now available for targeting CSCs. There are some high efficacy reports in cancer treatment using nanomedicine that have been demonstrated in the literature. Potent Breast CSCs killing ability by polyether ionophore salinomycin found to have better efficacy. Salinomycin resulted in the loss of Breast CSCs genes in patients [135] .Curcumin loaded nanoparticles emerge as a potent anticancer drug and detailed reviewed by Mimeault et. al. [136]. In Glioblastoma and medulloblastoma model Curcumin loaded NPs reported to potentially suppress CD133+ CSC [137]. Curcumin NPs has shown to block Hedgehog pathway in CSCs and reduced STAT3 and IGF levels. CSCs specific surface markers are utilised for targeting CSCs such as CD133 & CD44. In xenograft model of ovarian cancer treated with HA shell containing Bioconjugate of Paclitaxel in aqueous solution, HA shell endocytosis using CD44 marker on stem cell hindrance to cancer growth and metastasis was shown [138].

HA-NPs targeting in CD44+ Squamous cell carcinoma, Breast cancer also evaluated [139]. Lipoprotein like nanocapsules couple with thiolated anti CD133 monoclonal antibody targeting CD133 marker of CSCs has been known [140]. Bcr-Abl overexpressing chronic myeloid Leukaemia progenitor cells accumulate higher levels of low-density lipoprotein rather than non-CML cells. Imatinib was found to be used with LDL against leukaemia stem cells reported [141]. CD44+/CD24− cell isolated from Mcf-7 xenograft in nude mice treated with liposomal daunorubicin and tamoxifen resulting inhibition of Breast cancer stem cell significantly increases [142]. Mammosphere consisting MCF-7 cancer stem cell therapy using daunorubicin and quinacrine liposome which target mitochondria found to successful mitochondrial accumulation of drug and resulted high efficacy in tumour therapy. For treatment of Breast cancer relapse this mitochondrial targeting using nanomedicine provides new window to cancer treatment [143]. This liposome can accumulate in mitochondria and promote apoptosis of cancer stem cells by activating pro-apoptotic Bax protein. The liposome can penetrate deeply to the core of the spheroid and thus can kill cancer forming cells more efficiently. Kinase inhibition can fail various defence mechanisms used by CSCs by interacting with TME factor. Kinase inhibitor regorafenib and sorafenib are some multi kinase inhibitors are in use now in chemotherapeutic resistance.

9. Conclusion

Evidential studies raised huge attention to designing successful tumour therapy by targeting CSCs. Having multiple layers of self-defence strategies of CSCs makes it difficult for scientists. Conven-

tional cancer therapy failure can be explained by the properties and capabilities of CSCs. A new perspective about malignant cancer as a stem cell disease is emerging. Despite having wide attention to CSCs biology in tumour development and therapy resistance, detailed mechanisms about their ability to enter dormancy and contribution to relapse remain controversial. In order to develop successful therapeutic approaches, targeting CSCs specifically come as an important need of the hour. So, the knowledge about the specific marker for CSCs needs more lights.

Targeting CSCs using combination therapy against molecular pathways involving CSCs maintenance and its contribution towards Tumour growth could be a more promising strategy to improve cancer therapy. As the molecular mechanism involving chemo and radio resistance of CSCs requires further illustration to develop strategies to inhibit tumour relapse and progress. Nanoparticle mediated targeting to CSCs can improve drug efficacy. Certainly, nanomedicine can be an effective mechanism to reach out to CSCs specifically in the complex TME. Nonetheless, the Lack of an appropriate model of human tumour for experimental use which can accurately portray complex TME and cancer stem cell niche is a profound barrier in the way of tumour study.

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