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LUNG CANCER STEM CELLS:AN UPDATE

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ABSTRACT

Lung cancer is regarded as one of the most common cancers in the world with a worldwide occurrence of approximately 1.8 million cases and an estimated mortality of 1.6 million in 2012 alone.1 In the United States, there are approximately 2,25,000 new cases of lung cancer with over 1,60,000 deaths annually. Lung cancer is a very serious problem of the Indian subcontinent, especially in the lower socioeconomic subgroups. In India lung carcinoma is the 5th most common tumor and 2nd most common tumor in the males as per the ICMR [Indian Council Of Medical Research] registry of 2002.It accounts for 6.9% of new cancer cases detected each year.3

The importance of study of stem cells and cancer stem cells lays in CSC acting as prognostic and therapeutic markers. It is a known fact that there are innate stem cells present in the lining of the bronchial epithelium, at the carina and alveolar lining which help in regeneration of lungs post injury , however there are present similar cells which post driver mutations are christened CSC and help in cancer survival, growth and chemo resistance. CSC also known as "cancer stem-like cells" (CSLCs), or "tumor-initiating cells" (TICs) are heterogeneous cell population comprising of a small subpopulation of cancer cells with the property of self-renewal and differentiation. CSCs are thought to be responsible for cancer initiation, progression, metastasis, recurrence, and drug resistance.Important CSC under study in lung are, CD 133, ALDH 1, CD 44,ABCG2 etc.

Keyword: Lung, cancer, stem cell, markers, therapeutics , prognosis

Lung cancer is regarded as one of the most common cancers in the world with a worldwide occurrence of approximately 1.8 million cases and an estimated mortality of 1.6 million in 2012 alone.1 In the United States, there are approximately 2,25,000 new cases of lung cancer with over 1,60,000 deaths annually.2 Lung cancer is a very serious problem of the Indian subcontinent, especially in the lower socioeconomic subgroups. In India lung carcinoma is the 5th most common tumor and 2nd most common tumor in the males as per the ICMR [Indian Council Of Medical Research] registry of 2002.It accounts for 6.9% of new cancer cases detected each year.3

The major cause of development of such malignancy is the alkaloids and carcinogens present in the cigarette or bidi smoke being inhaled by the smokers as well as their family members passively, leading to predominantly squamous or the small cell variety of tumors. Other factors being implicated in pathogenesis of lung carcinoma are compounds like arsenic, asbestos beryllium, coal, coal tar, radon due to indoor exposure gamma radiations etc.

Patients can live with undetected lung cancer for years before it becomes apparent. Early lung cancer is largely asymptomatic and because of internalization of tumors the patients are not alerted by obvious physical changes. Squamous cell carcinoma takes around 8 years to reach a size of 30 mm when it can be commonly diagnosed so, by the time symptoms arise, the risk of metastasis is considerable. 6,7 Once symptoms appear they are often ignored by patients, delaying the diagnosis and treatment even further. The reasons for patient delay in diagnosis are poorly understood.

The high mortality of lung cancer is very largely because approximately 80% of patients with lung cancer have stage III or IV disease at presentation and are beyond therapeutic resection and care.8 Ample evidence of a prolonged preclinical phase in lung cancer has been seen. Detection of the tumor at an earlier stage improves the 5 year survival to around 60%.7 It is proven that the earlier the lung cancer is discovered, the better are the patient's chances of survival. Patients with radiograph-documented stage I lung cancer have a 5-year survival rate of 40 to 80%, whether discovered by screening or accident. However,

mortality in lung cancer worsens rapidly with advancing stage at the time of diagnosis. 9

Herein lays the importance of study of stem cells and cancer stem cells. It is a known fact that there are innate stem cells present in the lining of the bronchial epithelium, at the carina and alveolar lining which help in regeneration of lungs post injury , however there are present similar cells which post driver mutations are christened CSC and help in cancer survival, growth and chemo resistance.

STEM CELLS

*These are undifferentiated biological cells that differentiate into specialized cells and divide to produce more stem cells.*9They can be broadly classified into two types

1. Embryonic stem cells
2. Adult stem cells

There are two theories supporting the existence of a stem cell-

a) Cell division of two types SYMMETRICAL AND ASSYMETRICAL. Symmetrical division gives rise to two identical daughter cells with equal stem cells properties while asymmetric division gives rise to a daughter cell with stem cell like property and a progenitor cell with minimal regenerative capacity.10

b) Stem cells remain undifferentiated due to environmental changes in their particular niche.

A special population of stem cells known as cancer stem cells is piquing interest in the current world of cancer therapy and diagnosis and needs to be extensively studied.

CANCER STEM CELLS

CSC also known as “cancer stem-like cells” (CSLCs), or “tumor-initiating cells” (TICs) are heterogeneous cell population comprising of a small subpopulation of cancer cells with the property of self-renewal and differentiation. CSCs are thought to be responsible for cancer initiation, progression, metastasis, recurrence, and drug resistance. CSCs were first isolated in the 1990s, however, the concept dates back more than one and a half centuries ago.11 Bruce *et al*. in 1963 (12 observed that only a very small subpopulation of lymphoma cells could form *in vitro* colonies and initiate tumorgenesis in a xenograft transplant. In 1997 Bonnet and Dick provided the first compelling evidence for the existence of CSCs when they. 13 Isolated a subpopulation of CD34+CD38– acute myeloid leukemia (AML) cells capable of initiating hematopoietic malignancy in mice. Many other organs have been used since then, to harvest CSC from like, the brain ,head and neck ,breast , lung , liver , colon , pancreas , ovary and prostate .

Many current studies based on the model in which states that CSC markers are either surface biomarkers or enzymatic activity, are being tested now. Also it is being seen that tumor CSC's have a tendency of forming spheroids with the cells lying in a <5 mm niche with suitable microenvironment.14

Despite this obvious contradiction in the role CSC it is still being researched upon as its use in cancer therapeutics is being herald as a new breakthrough in the world of chemotherapy. Regardless of whether CSCs all stably or transiently express biomarkers; have a common or diametric origin; are a rare or abundant population, a greater insight into their mechanism of development, function, and interplay with the tumor microenvironment (TME) will likely result in the development of novel molecular targeting strategies.15

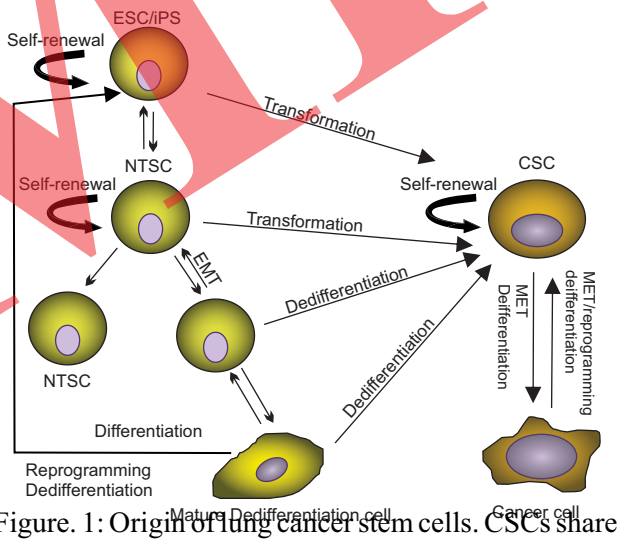

Figure. 1: Origin of lung cancer stem cells. CSCs share many properties of tissue specific stem cells, including phenotype, self-renewal capacity, and multipotency.

ESC or iPS cells have self-renewal ability and are programmed towards functioning as NTSC. Response to intrinsic or micro environmental signaling cues may result in CSC development from PC, mature differentiated cells, cancer cells by phenotypic conversion, and NTSC by transformation. The dedifferentiation process is orchestrated by EMT and/or induction or depression of reprogramming processes. ESC, embryonic stem cell; iPS, induced pleuripotent stem cells; NTSC, normal tissue-specific stem cells; PC, progenitor

STEM CELL NICHE

Microenvironments are defined as the sum total of cell-cell, -extracellular matrix (ECM), and -soluble factor interactions, and the physical states and

geometric constraints that a cell may experience.16 The stem cell niche is a specialized microenvironment where normal cells and CSCs reside and it is composed of a heterogeneous population of cells that provide normal stem cells with signals to proliferate, differentiate, and undergo asymmetrical cellular division.15 Extrinsic cytokines from the niche and the intrinsic genetic programs within the stem cell regulate the normal stem cell activity.16

LUNG STEM CELLS

Researchers have observed stem cells in mice and have studied the cellular response to lung injury to find cells with stem-like characteristics, such as self-renewal and multi potential,17-18 Lung stem cells have been found to exist in functionally and anatomically distinct locations including branch points of airway tubes and junctions between the conducting airway and gas exchange regions.

A brief description of various points in the airway having stem cells can be seen below

Cellular turnover in the adult trachea is very low.19 Epithelial injury elicits a rapid response of proliferation in surviving cells, except ciliated cells, 20 to repair the tissue. It has been seen that adult basal cells are capable of differentiating and proliferating to form a fully differentiated mucociliary and functional airway epithelium 21,22 and they form heterogeneous spheres capable of both self-renewal and differentiation.23 Another point of stem cell origin is the nonciliated Clara cells which detoxify and protect bronchiolar epithelium. Some 24 experiments on murine lung CCSP-expressing cells that exhibited a number of stem cell-like properties including expression of stem cell markers CD44, CD133, and Sox2; bronchosphere colony formation; and self-renewal capacity. While the specific cell of origin that gives rise to small cell lung carcinoma (SCLC) remains to be elucidated, it is important to note that SCLC predominately localizes to the midlevel bronchioles and is associated like PNECs with primitive neuroendocrine features, such as expression of the neuropeptide Calcitonin-Gene Related Peptide (CGRP), 25 potentiating that PNECs may be the origin of SCLC.Distal airway stem cells (DASCs) expressing p63 that generated alveoli *in vitro* and*in vivo* following lung injury induced by infection.26

LUNG STEM CELL MARKERS

CSC markers are widely accepted in CSC research for isolation of human CSCs in multiple solid tumors 27,28,29. Despite an increase in trend of stem cell research in other solid tumors, identification and validation of lung CSCs has largely been obstructed by the complexity of the disease and a deficiency in understanding the hierarchical structure of lung epithelial stem cells.

Cd133

CD133 (*Prom1*), a cell surface glycoprotein, was first identified as a useful marker in the selection of hematopoietic and neural stem cells. 30 A study 31 found a rare undifferentiated cell population expressing CD133 in both NSCLC and SCLC specimens. These cells grew indefinitely as tumor spheres, generated tumor xenografts phenotypically identical to the parental tumors, and were resistant to conventional chemotherapy. Other groups confirmed these findings as well as demonstrated both *in vivo* and *in vitro* that long-term chemotherapy exposure could enrich for CD133+ cells in lung cancer. 32,33 These CSC have been found to participate in tumor initiation,progression, promote vasculogenesis ,metastasis and tumor proliferation.34 The significance of CD133 expression as a prognostic marker in NSCLC has been controversial.35,36,37 Some scientists 38 observed that CD133 expression was significantly correlated in NSCLC with pathological stages II, III and IV and was an independent factor for poor prognosis. Salnikov *et al*. 39 have reported that CD133 was indicative of a resistant phenotype and may be used as a predictor for efficacy of cytotoxic therapy, but it did not represent a prognostic marker for NSCLC. CD133 has been described as a CSC marker in other tumors and its expression correlated with chemoresistance to etoposide and increased tumorigenicity accompanied by increased expression of CD133 in human SCLC lung biopsy samples following chemotherapy. In addition, CD133 positive cells express increased neuro peptide receptors for gastrin releasing peptide and arginine vasopressin (40). In another study, Eramo *et al*. showed that CD133 is also a useful marker in SCLC (41) Roudi *et al*. CD44 and CD24 Cannot Act as Cancer Stem Cell Markers in Human Lung Adenocarcinoma Cell Line A549

R Roudi et al.Cell Mol Biol Lett 19 (1), 23-36. 2013 Dec 23.also investigated CD133 and ALDH1 stem cell marker expression in lung cancer patients and found that ALDH1 and CD133 had higher expression in NSCLC compared to SCLC. High expression of ALDH1 and CD133 could be considered to be a CSC marker in some lung cancer subtypes such as SCC and ADC (42). Jiang *et al*. demonstrated that achaete-scute complex homolog 1 (ASCL1) regulates ALDH1 and CD133 and that CD133high- ALDH1high-ASCL1high subpopulation had CSC features *in vitro* and *in vivo* (43

Wang *et al*. characterize a SP fraction in the H446 SCLC cell line and found 6.3% of SP cells by flow cytometry. They also found that SP cells were able to form tumor spheres better than non-SP cells. mRNA expression of the CSC markers ABCG2, CD133 and nucleostemin was analyzed and found to be 21.6, 7.1 and 1.02 higher than in non SP cells, respectively.(44) SP cells have a greater ability to form tumors when compared with non SP cells and showed better proliferative ability and tougher viability when treated with drugs.

Some scientists state that an increase of stem cell markers urokinase plasminogen activator receptor (uPAR) and CD133 compared with parental cells was seen in SCLC in

transplanted mice.(45). Gutova *et al*. reported that SCLC cells positive for uPAR were resistant to conventional chemotherapy and speculated that they contain a CSC subpopulation (46). Another worker studied CD133 and CD87 like CSC markers in a panel of six SCLC cell lines, of which the SBC-7 cell line showed the highest expression levels of both markers.(47) The researchers therefore concluded that CD133 and CD87 are inadequate CSC markers inSCLC (48)]

Some scientists identified a small cell group in AML patients capable of initiating leukemia in a mouse model after injection [49]. CD133 mainly gained interest after experiments showing that a CD133+ subpopulation in a brain tumor had stem cell properties in vivo. After transplantation of only about 100 CD133+ cells into immune deficient mice, the exact same tumor was induced, whereas this was not the case for the same number of CD133- cells [50]. The capability of a minor CD133+ cell fraction to induce the identical tumor after transplantation into immune deficient mice holds true for colon carcinoma [37]. Consistently, CD133 expression in high levels was shown for telomerase reverse transcriptase immortalized primary nonmalignant and malignant tumor-derived human prostate epithelial cell lines [38]. Few studies showed enhanced[CD 133] as a result of changes in receptor glycosylation [51] and alternative splicing of the extracellular domains of human CD133 which may affect presence as specific epitopes [52]

Workers used A549 cells and found a 4% expression rate in mean. After enrichment using an anti-human CD133 PE antibody expression level of CD133 was about 40% [53]. Furthermore,two distinctive groups found out that CD133+ colon carcinoma cells were able to initiate tumor growth, which was not visible for CD133_ cells [53]. Researchers still struggle to entirely understand the role of CD133. However this process is also supported by progress in protein engineering capable to more reliably bind CD133 related epitopes. New therapeutic anti-CD133 antibodies are useful only if their ability to bind CSC is indisputable.

Fortunately, this can be readily tested using the antibody to sort CD133+ cells and then injecting

them as flank tumor xenografts. Enriched populations are expected to grow fast and larger while the diminished fractions grow more weakly and slowly.

CD44, a multifunctional transmembrane glycoprotein, which is commonly expressed in embryonic , hematopoietic , mesenchymal , and certain epithelial CSCs (54). CD44 binds hyaluronic acid, an abundant polysaccharide in stem cell niches , to facilitate adhesion, differentiation, homing, and migration within normal and CSC niches. In lung cancer elevated CD44 expression was initially detected on specific differentiation phenotypes, including activated type II pneumocytes, squamous metaplasia, and NSCLC cells suggesting it may play a role in the progression of disease 54. It has been 55 demonstrated a subpopulation of CD44+ NSCLC cells were cisplatin-resistant and capable of spheroid body formation, *in vivo* tumor initiation. Collectively, CD44 is poised to be a key player in identifying CSCs due to its innate ability to regulate adhesion, differentiation, homing, and migration. A study established a panel of lung cancer cell lines from primary tumors and characterized a small subpopulation strongly positive for CD44 (CD44high), with the main population being weakly positive or negative for CD44. Co-expression of CD90 (CD90+) further narrowed down the putative stem cell population. This CD44 and CD90 positive subpopulation showed mesenchymal morphology, increased expression of the mesenchymal markers vimentin and N-cadherin, increased Mrna levels of the embryonic stem cell-related genes Nanog and Oct4, and resistance to irradiation compared with other subpopulations. The CD44high CD90+ subpopulation is therefore a good candidate for a CSC marker (56)Some scientists have also studied the stem cell marker and cell adhesion molecule CD44 in different histological subtypes of lung cancer, analyzing 195 lung tumor samples, including 37 SCLC samples, by immunochemistry (IHC). Univariate analysis demonstrated that CD44 expression was higher in NSCLC compared to SCLC. In NSCLC, a higher level of CD44 expression was found in squamous cell carcinomas (SCC) compared to ADC. Higher CD44 expression correlated with higher grade tumors which in turn correspond to poor prognosis in SCC, and the lower level of CD44 expression was more often found in well differentiated ADCs. Also, high CD44 expression was associated with decreased levels of the proliferative marker Ki67 (49). Urokinase plasminogen activator (uPA) and its receptor uPAR play an integral role in regulating pathways important in cell migration and invasion.57 A study done on subpopulation of SCLC cells from multiple cell lines demonstrated multidrug resistance, clonogenicity, and co-expressed the putative CSC marker CD44.58

ALDEHYDE DEHYDROGENASE (ALDH)

ALDH detoxifies cells by oxidizing intracellular aldehydes and is known to play a role in differentiation of normal stem cells.59 On analysing two aldehyde dehydrogenase isozymes, ALDH1A1 and ALDH3A1,it was seen that they are overexpressed in NSCLC cells, atypical pneumocytes, and normal pneumocytes with chronic carcinogen exposure .Scientists 60) further advanced the ALDH association by demonstrating an ALDH+ subpopulation from multiple NSCLC cell lines had enhanced tumorigenicity, clonogenicity, and Notch signaling dependent self-renewal capacity. Similarly, another worker 61 demonstrated that an ALDH subpopulation (ALDHhigh) of NSCLC cells had stem cell-like qualities including increased spheroid formation and metastatic activity. Unlike CD133 and CD44,

ALDH is useful in tumor staging and prognosis 62 . NSCLC patients harboring tumor cells overexpressing ALDH1A1 have significant resistance to EGFR tyrosine kinase inhibitors and chemotherapy drugs 63 likely resulting in their poor clinical outcome 64. Aldehyde dehydrogenase (ALDH) is a candidate marker for lung cancer cells with stem cell-like properties. Immunohistochemical staining of a large panel of primary non-small cell lung cancer (NSCLC) samples for ALDH1A1, ALDH3A1, and CD133 revealed a significant correlation between ALDH1A1 (but not ALDH3A1 or CD133) expression and poor prognosis in patients including those with stage I and N0 disease. Flow cytometric analysis of a panel of lung cancer cell lines and patient tumors revealed that most NSCLCs contain a subpopulation of cells with elevated ALDH activity, and that this activity is associated with ALDH1A1 expression. Isolated ALDH(+) lung cancer cells were observed to be highly tumorigenic and clonogenic as well as capable of self-renewal compared with their ALDH(-) counterparts. Expression analysis of sorted cells revealed elevated Notch pathway transcript expression in ALDH(+) cells. Suppression of the Notch pathway by treatment with either a γ-secretase inhibitor or stable expression of shRNA against NOTCH3 resulted in a significant decrease in ALDH(+) lung cancer cells, commensurate with a reduction in tumor cell proliferation and clonogenicity. Taken together, these findings indicate that ALDH selects for a subpopulation of self-renewing NSCLC stem-like cells with increased tumorigenic potential, that NSCLCs harboring tumor cells with ALDH1A1 expression have inferior prognosis, and that ALDH1A1 and CD133 identify different tumor subpopulations. Therapeutic targeting of the Notch pathway reduces this ALDH(+) component, implicating Notch signaling in lung cancer stem cell maintenance(65). Thus, ALDH1A1 is a potential tumor suppressor whose downregulation promotes carcinogenesis, especially in cases of ADC. The finding that the restoration of ALDH1A1 expression markedly suppressed the growth of some lung cancer cell lines supports this possibility. Other studies have found that ALDH1A1 expression was not detected in 40% (12/30) to 56.1% (38/66) of NSCLCs [66], and in 35% (7/20) of lung ADCs [67]. Among lung ADCs, its level was reported to be lower in invasive tumor than in premalignant or non-invasive ones . Also, ALDH1A1 expression was reported to be reduced in other malignancies: 76.9% (970/1287) of colorectal cancers , 21.1% (4/19) of pancreatic cancers, and 70.8% (46/65) of ovarian cancers [68]. It has been seen that ALDH proteins (ALDH1A1 and ALDH1A2) oxidize retinol to synthesize retinoic acid and induce growth arrest and differentiation [69, 70]. ALDH1A2, a family member closely similar to ALDH1A1, was reported to be epigenetically silenced through the hypermethylation of its promoter region in prostatic cancer cells, and forced expression of ALDH1A2 markedly suppressed the growth of the prostatic cancer cells, implying ALDH1A2 to be a tumor suppressor, loss of which could result in an impairment of retinoic acid synthesis to interfere with cellular differentiation and promote the progression of carcinogenesis [71].

Aside from such a suppressive role, studies in vitro demonstrated that ALDH activity or ALDH1A1 protein expression was strong in stem cell fractions in a variety of malignancies including lung cancer, and suggested that ALDH1A1 could participate in the maintenance of cancer stem cells. Also, silencing experiments in vitro showed that a forced reduction of ALDH1A1 attenuated growth and migration in some lung cancer cell lines, suggesting an oncogenic role [72].

Interestingly, the downregulation of ALDH1A1 expression occurred with a significantly higher incidence in smokers than non-smokers. It is possible that this association is a result of smoking and also a mechanism promoting smoking-related carcinogenesis. Thus, ALDH1A1 might act as a tumor suppressor especially in smoking-related carcinogenesis.DNA hypermethylation could be a possible mechanism of ALDH1A1 downregulation in NSCLCs.

Retrospective study of expression of CSC makers such as Caveolin, Notch, CD44, CD166, SOX2, ALDH1 and Musashi 1 in patients who underwent surgical resection of SCLC (n=60) and large cell neuroendocrine carcinoma (LCNEC) (n=45). They found a difference between SCLC and LCNEC, with regard to both SOX2 (55% *vs*. 27%, P=0.003) and CD166 (27% *vs*. 47%, P=0.034). ALDH1 expression was similar in SCLC and LCNEC (67% *vs*. 73%, P=0.46) and ALDH positive patients had significantly worse recurrence-free survival (RFS) and OS rates compared with ALDH negative patients(5-year RFS: 39% *vs*. 67%, P=0.009; 5-year OS: 50% *vs*. 79%, P=0.021). A multivariate analysis revealed that positive ALDH expression was an independent unfavorable prognostic factor with regard to both RFS and OS (73)

To name a few other CSC's in lung carcinogenesis we have CD166, also known as Activated Leukocyte Cell Adhesion Molecule (ALCAM), which has been seen to be involved in angiogenesis, differentiation, homing, and maintenance of hematopoietic stem cells. Another worker 74 identified CD 166 in NSCLC patient tumor tissues a subpopulation.

ABCG2 is an ATP binding cassette transporter which pumps chemotherapeutic drugs out of the cell resulting in decreased concentration and hence resistance. Recent studies have shown that this marker expressed in lung cancer stem cells is responsible for resistance. However it has been seen to have a cytoprotective role as well in cardiac cells. Overall it contributes to preservation of CSC by reducing oxidative stress indirectly and increasing its survival(75).

Another marker BMI1 polycomb ring finger oncogene

(BMI-1) is involved in axial patterning, haematopoiesis, and cell cycle regulation 76. It is known to have a critical role in regulating self-renewal and maintenance of normal lung epithelial and BASCs and CSCs.

Podocalyxin-like protein 1 (PODXL-1) is a glycosylated sialomucin and known marker of embryonic, mesenchymal, and hematopoietic stem cells and has been described in multiple human malignancies. Proximal bronchi and SCLCs support the role of PODXL-1 as a potential CSC marker in SCLC.(77)

No clear distinction of definitive CSC for lung carcinoma has been seen till now. Intratumoral variation in CSC markers strongly suggests that phenotypic markers may not be a reliable identification tool, but rather cellular state may play an important role in defining a CSC. Researchers state that CSC pool populations are highly heterogeneous between tumors and within individual tumors(78).

Pathways being studied in pathogenesis of lung cancer connected to the genesis of these CSC are Hh ,Wnt and Notch pathway. However since this review focuses on CSC we won't discuss it in detail.(79)

CSC targeted therapies

Therapeutic failure is commonly associated in multiple malignancies with resistance to chemotherapy and radiotherapy. The CSC hypothesis accounts for these observed patterns of recurrence and metastasis. CSCs are known to be more resistant to conventional chemo- and radio-therapy compared to non-CSC populations. The mechanisms by which CSCs are resistant to conventional therapies is very diverse, compounding an already difficult scenario. Some of these mechanisms include slowing cell cycle kinetics, efficiently employing mechanisms of DNA repair, intrinsic expression of anti-apoptotic proteins, resistance to oxidative or DNA damage, residence in hypoxic niches, and upregulation of expression of multidrug resistance type membrane transporters.(80).

However the CSC hypothesis has generated a lot of excitement for the discovery and development of therapeutics targeted towards the CSCs. Successful pre-clinical and clinical trial studies have employed strategies of targeting CSCs via use of CSC surface markers, inhibition of developmental stem cell pathways, and ablation of CSC niches.

LIMITATIONS

The CSC hypothesis, however, has come under scrutiny and remains controversial. For example, the theory that tumor growth must be initiated by a rare CSC population has been challenged several times. Some workers 81 have demonstrated that when lymphomas and leukemia's of mouse origin are transplanted into histocompatible mice, a very high frequency of tumor cells (1 in 10) can seed new tumor growth. Additionally, skeptics of the hypothesis criticize the use of surface biomarkers or enzymatic activity for the identification of CSCs and their lack of utility in therapeutic exploitation. The validity of their claim is proved by both the heterogeneity of the proposed CSC markers as well as in the recent discovery of the dynamic state of marker expression on CSCs. Another limitation to this hypothesis is that the markers that have been identified are not specific to CSC populations, but rather are also abundantly expressed on healthy tissue. Another important finding contradicting the role of CSC in tumorigenesis is the fact that presence of these CSC populations in tumor has been seen to be transient .(82)

CONCLUSIONS AND PERSPECTIVE

Despite all the limitations and challenges the role CSC it is still being researched upon as its use in cancer therapeutics is being herald as a new breakthrough in the world of chemotherapy. A greater insight into their mechanism of development, function, and interplay with the tumor microenvironment (TME) will likely result in the development of novel molecular targeting strategies

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