Oncology
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1 (51) 2020: 001-007 • REVIEW ARTICLE

Hallmarks of cancer stem cells in men associated cancers: a review

Sreedhar Surampudi[1], Satyanarayana Swamy[1], Satyanarayana Rentala[2], Sivarama Prasad Darsi[1]

[1] Department of Biotechnology, GITAM Institute of Science, GITAM University, Gandhi Nagar, RushiKonda, Visakhapatnam, 530045, Andhra Pradesh, India
[2] Department of Health Informatics and Analytics, The Apollo University, Chittoor, 517 127, Andhra Pradesh, India

SUMMARY

Failure of conventional therapies is the root cause for the progression of cancer. Intrinsic revelation states that Cancer metastasis is due to subclonal diversification of normal cells into subsets of stem-like cells. These cells can be termed as stem cells of cancer or cells that initiate cancer. These diversified cells can differentially activate and escape from the resistant mechanisms causes tumor heterogeneity. Because of these metastatic fuelling which is malignant, it expresses its persistence towards treatment and provokes drug-resistant recurrence. Annihilation of these subsets of stem-like cells in cancer tissues has now become a prime objective to develop and design novel classes of anti-cancer therapeutics to improve clinical efficacy. In this review, the properties and hallmarks of different cancer stem cells that are responsible for disease recurrence and metastases which are elusive targets for present oncotherapies were presented.

Key words: cancer stem cells, tumor heterogeneity, malignancy, clinical efficacy, oncotherapies

Abbreviations: ABCG: ATP Binding Casette Transporter; ADC: Adenocarcinoma; AFP: Alpha Feto Protein; AKT: AKT8 Virus Oncogene Cellular Homolog, Serine/Threoning Kinase; ALDH: Aldehyde Dehydrogenase; ALK: Anaplastic Lymphoma Kinase; APC: Adenomatous Polyposis Coli; Atg: Autophagy Related Proteins; BCSC: Breast Cancer Stem Cells; Bmi: B-cell specific Moloney Murine Leukemia Virus Integration Site; BMI: Body Mass Index; BRAF: Rapidly Accelerated Fibrosarcoma of Murine Associated Viral Onocgene Homolog B; CD: Cluster of Differentiation; CET: Clonal Evolution Theory; CRC: Colorectal Cancer; CSC: Cancer Stem Cells; EGFR: Epidermal Growth Factor; EMT: Epithelial Mesenchymal Transition; EpCAM: Epithelial Cell Adhesion Molecule; ERB: Pan Eryhthroblastic Leukemia Viral Oncogene Homolog; GB's: Glio Blastaoma; HIF: Hypoxia Inducing Factor; HNSCC: Head and Neck Stem Cell Carcinoma; ITGB: Integrin Mediated Cell Adhesion; JAK: Janus Kinases; KEAP: Kelch like ECH Associated Protein; KRAS: Kirsten Rat Sarcoma; LCSC's: Liver Cancer Stem Cells; LGR: Leucine Rich Repeat Containing G Protein Coupled Receptor; Lrig: Leucine Rich Repeats and Immunoglobulin Like Domains; LSQCC : Squamous Cell Carcinoma; MAPK: Mitogen Activated Protein Kinase; MSI: Micro Satellite Inhibition; MSS: Micro Satellite Stability; Myc: Myelocytomatosis Oncogene Cellular Homolog; NF: Nuclear Factor; NPSC'S: Neutral Stem Cell Progenitors; NSCLC: Non Small Cell Lung Cancer; OCT: Octamer Binding Transcription Factor; PCa: Prostate Cancer; PI: Phospho Inositide; PTEN: Phosphatase and Tensin Homolog; RAF: Rapidly Accelereated Fibrosarcoma; RAS: Rat Sarcoma; RET: Receptor Tyrosin Kinases for Members of the Gndf Members of Extracellular Signaling Molecules; RIT: Wild Type GTP Binding Protein; ROS: Reactive Oxygen Species; RTK: Receptor Tyrosine Kinase; RUNX: Runt Related Transcription Factor; SCLC: Small Cell Lung Cancer; SOX: Sry Box Transcription Factors; SSEA: Stage Specific Embryonic Antigen; STAT: Signal Transducer and Activator of Transcription Proteins; TGF: Tumor Growth Factor; TP53: Tumor Progression 53; VEGFR: Vascular Epidermal Growth Factor; Wnt-Catenin-Beta: Wingless Integrin Catenin Beta Pathway

Address for correspondence:

Sreedhar Surampudi, Department of Biotechnology, GITAM Institute of Science, GITAM University, Gandhi Nagar, RushiKonda, Visakhapatnam, 530045, Andhra Pradesh, India, email: satyamswami@gmail.com

Word count: 5481 **Tables:** 00 **Figures:** 02 **References:** 62

Received: - 03 March, 2020

Accepted: - 16 March, 2020

Published: - 26 March, 2020

INTRODUCTION

Day by day one of the increased global burden threatening diseases is Cancer. Nearly, on the estimation of about 14.1 million cancer cases were recorded in the world by 2012. 7.4 million Cases were seen in men and 6.7 million in women. This number is expected to increase by 24 million by 2035. Lung cancer, the most common cancer which contributes 13% of the total number of new cases was diagnosed in 2012 worldwide. Likewise, Breast cancer (women) the second most common cancer (1.7 million) and Colorectal cancer, the third most common cancer (nearly 1.4 million) new cases were diagnosed in 2012 [1].

Cancer probabilities are increasing day by day which is the second common disease in India with a maximum mortality rate of 0.3 million deaths per year [2]. Failure expression of clinical therapies in treating cancer patients is due to recurrence or relapse. Recurrence properties extrapolate Intra and inter tumor heterogeneity of cancer between the same or different cancer patients or within the tumor of different types. The facial expression of cancer doesn't show any correlation between phenotype and genotype. Cancer initiation and progression rely on the theory of Clonal evolution which is a neutral drift dynamic theory [3, 4]. The dynamicity operates through selective sweeps with the help of drivers and passengers that are carried along during the development of cancer [5].

A single tumor biopsy sample doesn't give insights into the tumor characteristics or its genomic landscape. Intratumor heterogeneity can be elucidated through the evaluation of topologically distinct regions with existing techniques that determine clonal heterogeneity [5, 6]. Tumor behavior and response to therapy explain the inter tumor heterogeneity even though the tumor origin is from the same organ [7]. Intra and inter tumor progression will also be influenced by a group of a subset of cells named as Cancer Stem Cells (CSC's) having self-renewal with differentiation [8]. These stem cells explain the differences between different tumor cells in the form of gradient differentiation between them [9, 10].

In this scenario, studying tumors and their heterogeneity considering the theory of CSC in different cancer types explains the insights of targeted clinical cellular therapy. Here, in this review, an overview of several aspects that contribute CSC based tumour heterogeneity based on their cell surface markers has been presented. We also discussed the nature of

intra- and inter-tumor heterogeneity that draws parallels that might contribute to our basic understanding of two sides of the cancer Clonal Evolution Theory (CET) that counteracts clinical context in exposing the cell surface markers as their hallmarks of individual cancers.

Cancer and CSC's

Cancer and its progression are connected to the random dysregulation of the cellular genome and metabolome flux density. This dysregulation shows its impact in 8 stages. Primary and major recognizing factor in cancer is to bring alterations in programmed cell death mechanisms that lead to resistance to cell death. The thermodynamic

balance of cellular/tissue bioenergetics is another important aspect that is required to carry out normal threshold metabolomics flux density. We can observe the deregulating cellular energetics in cancer cells. Counteracting the cellular energetic, it shows its impact on several cells and its balance (synthesis and proliferation). Deregulation of bioenergetics shows its impact on sustaining cell proliferation. Next to this mechanism, there is a drastic drift on the deficit of evading growth suppressors. Due to sustained proliferation, a random effect on the dynamicity of the DNA replication can be seen. This enables replicative immortality (Figure 1). Due to the deregulation of the genome and metabolome, it activates invasion thereby leads to the condition called metastasis and inducing angiogenesis to channelize the propagation of the accumulated group of cells. This unstable homeostasis induces genomic instability and mutations [11].

Potentially, all normal cells may not be converted into cancerous cells. Not necessarily, all cancerous cells to be converted into a subset of cells as cancer stem cells. The concept of these CSC was first proposed 45 years ago as complete analysis hasn't been done until the development of advanced research tools. The extraction of CSC evidence was depicted based on the hematological malignancies; its potentiality has been revealed on the characteristic features of embryonic stem cells. Hence, each organ has its property based stem cells; hence different cancers have different CSCs thereby in different cancers has different CSCs. These CSCs can also be referred to as progenitor cells that are the main possible cells for the tumor growth and relapse of cancer [12-15]. The nature of the tumor is not the same as in tumors present in the same person as the same type of different persons.

CSCs are self-renewing, totipotent cells resemble the characteristic feature of normal cells. These groups of a subset of cells will show its propagation either be symmetric or asymmetric division. The descendants of these cancer stem cells progeny constitute the bulk of the heterogeneous tumor. Irregular symmetric or asymmetric cell division maintains the number of CSCs inside the tumors. Studying the nature of these tumors of different types will help us to understand the basic interactive neutral drift dynamic changes. These studies will also make us to understand the counteracting metastatic proclivity building with a programmed clonal evolution sequence [16-20].

Lung cancer: An estimated responsible for 85% of all cancers is lung cancer and smoking is one of the possible reasons. In consideration of taking the standardized data, 1.8 million cases were diagnosed by 2012. Hungary stood highest in the diagnosis of the highest number of lung cancers and 58% have occurred in less developed countries. Ferlay's studies state that the incidence of the highest lung cancer is seen in North America, Europe and lowest in Africa, Latin America and the Caribbean was observed. The percentage of a higher probability of lung cancers in the world is seen in men than women. The higher incidence in women is also showing reflection towards the type of tobacco consumption based on socioeconomic status and ethnicity.

Lung cancers, Non-Small-Cell Lung Cancer (NSCLC) or Small-Cell Lung Cancer (15%) (SCLC) constitutes about 85% of deaths. Out of these, two histological predominant phenotypes are Adenocarcinoma (ADC) and Squamous Cell Carcinoma (LSQCC) [11]. In comprehensive molecular profiling, lung adenocarcinoma studies were conducted in 230 ADC patients. In this study, on average 62% display active mutations in known driver oncogenes (EGFR, KRES, BRAF mutations, ALK, ROS1, and RET fusions) and are recurrent genetic alterations. 38% are without apparent mutations (RTK/RAS/RAF). It has shown that TP53, KEAP1, NF1 and RIT1 mutations enriched

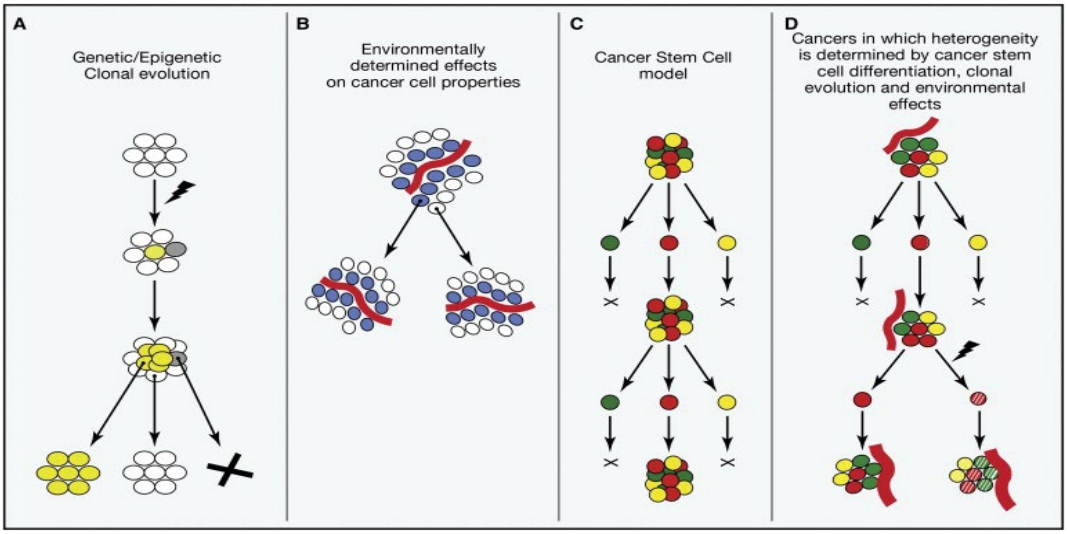

Fig. 1. Sources of heterogeneity within cancer

in the oncogene-negative group of ADC's [11, 21]. Activating and recurrent mutations influences the key alterations in the biochemical pathways. 76% of alterations can be observed in RTK/RAS/RAF pathway and the remaining are PI3K-mTOR pathway (25%), p53 pathway (63%), cell cycle (64%), chromatin and RNA splicing (22%) respectively [22].

Genetic alterations or mutations of NSCLC/SCLC usually can carry out by three methods as trunk, branch, and driver. Trunk mutations usually linked with a ubiquitous pathway (linked to the apoptotic mechanism). Branch will at specific regions and driver is only in specific cells. Mutations linked with trunk and branch or trunk with a driver may lead to the recurrence or relapse of cancer [23-25]. Recurrence is purely associated with cell propagation through angiogenesis may be regretted during the primary stage due to inhibition by drug resistance. Later stages of recurrence or long term angiogenic propagative cells are drug-resistant as these are having lung-specific subset of the population known as CSC's. These cells have specific recognition markers along with ALDH like CD133+ (bertholi and coworkers), ABCG2 (cisplatin-induced chemotherapy study) and NOTCH1 (chemotherapy resistance). The propagation of the tumors can be recognized by the markers like ITGB4 and CD24+ (based on the transplantation studies) [26-40].

A strong rationale for new therapeutic options has been developed to characterize lung cancer molecular abnormalities provided with understanding the mechanisms of drug resistance. The subclonal combinatorial mutations increase the complexity of lung cancer genomes elucidated through sequencing studies that support the heterogeneity of tumors at the cellular level. Molecular studies performed in lung tumors during treatment have shown the phenomenon of clonal evolution and thus supports the occurrence of temporal tumour heterogeneity.

Prostate cancer: As per the studies done by a section of cancer surveillance, On-screen, positive prostate-specific antigen without signs and symptoms of the disease is increasing in men at which 1.1 million and above cases were recorded by 2012. The highest incidence can be seen in Martinique, Norway, and France as next followed by 68% can be seen in developed countries. Lowest incidence can be seen in Asia and Africa.

Normal Prostate stem cells are present in epithelial cells of Basal, luminal and neuroendocrine cells of prostate tissues. Basal cells express high molecular weight proteins whereas luminal cells that are terminally differentiated express low molecular weight proteins. Luminal cells secrete prostate-specific antigen and prostate-specific alkaline phosphatase into the glandular lumen through androgen dependant cascade. Secretory luminal and neuroendocrine cells express and secrete neuropeptides, synaptophysin, and chromogranin A which are not androgen dependant. Many studies elucidate the markers present in the prostate stem cells of three regions and have been depicted in the below-shown diagram (Figure 2). Multitude markers participate in tumor progression, self-renewal capacity, metastatic colonization and growth and recurrence and therapeutic resistance [41]. The common signaling pathways operate are NOTCH, HedgeHog and WNT.

Normal Stem cells of prostate tissues operate microenvironment of non-neoplastic activities like the cellular growth, signal response towards pre-apoptotic cell death mechanism, minimal chromosomal aberrations, controlling intensified cellular metabolic and genomic hyperactivity. Not necessarily, all the normal prostate stem cells need not be converted into prostate cancer stem cells. Conversion into primary PCa CSC's are due to loss of response towards the cellular preapoptotic signals, cell-cell contact inhibition, DNA repair splicing mechanisms, hypermutations due to chromosomal aberrations and so on. Not all prostate cancer stem cells express markers present on prostate stem cells. Hence, origin and conversion into prostate cancer stem cell as becoming the main conversation [42]. Malignancy development from normal stem cells is the primary concept as in mouse models and it has been shown that basal epithelial cells are more potent in the conversion of PCa even though both basal and luminal will participate. During castration, basal cells will be under control for the regular differentiation mechanism but luminal cells are completely lost due to the apoptotic cell death mechanism. Hence castration becomes a weapon for the identification of the altered mechanistic aversion of prostate cancer from stem cells. After Androgen replacement therapy,

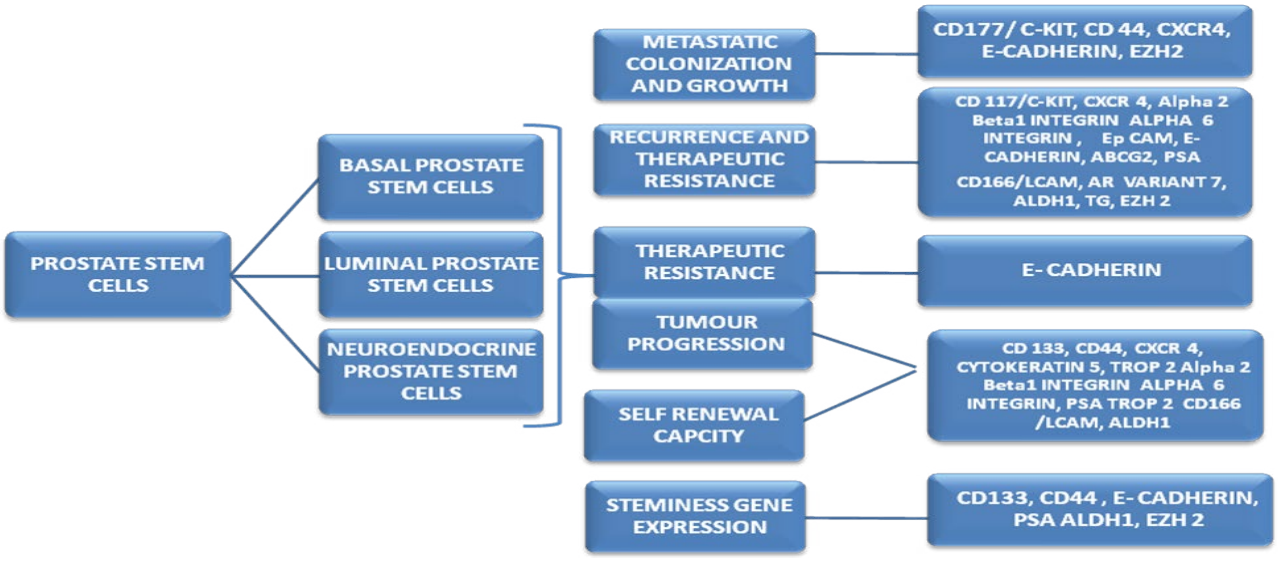

Fig. 2. Makers of prostate cancer stem cells

some of the luminal progenitor cells will survive and progressed towards prostate cancer which can be considered as castration-resistant phenotypically [41, 43-47].

Liver cancer: Liver, an important regenerative organ that has been affected due to cancer is the sixth common cancer in the world. Annually 1 million or fewer new cases are diagnosing at which 90% of the cases are hepatocellular carcinomas. Hepatitis infection is considered one of the causes on one side and due to alcohol, preservative foods, contaminated aflatoxin, and body fatness are on another side for getting liver cancer. Liver cancer by Hepatitis B is due to damaging cells and hypermutations in DNA whereas in hepatitis c indirect effect mediated by liver cirrhosis. Sources, methods, and patterns on cancer state that the greater incidence of liver cancer can be seen in magnolia, Lao PDR and Gambia. The lowest incidence can be seen in Europe, Latin America, and the Caribbean.

The best strategic treatment to treat liver hepatocellular carcinoma is only surgery. Other than this other oncotherapies are radio and chemotherapy. Molecular mimicry and altering genetic predispositions lead to recurrence or relapse of liver cancer. Insight vision of this relapse shows the development of a subset of cells that are responsible for tumor progression termed as Liver Cancer Stem Cells (LCSC's). Usually, stem cells will lead its leadership during injury or any damage to bring down the environment into control.

LCSC's resembles stem cell properties and perpetuates tumor progression having its characteristic representations. Ma et al., first identified stem cells in hepatocellular carcinoma characterized by cell surface marker CD 133 in HCC cell lines and xenograft tumors [48]. Many studies have been carried out for the prognostic cell surface marker of LCSC. CD133+, CD90+, CD44+, OV 6, ABCG 2 and ALDH 1 are the markers that have been identified on one side which has been slashed with the discovery of another marker EpCAM+. AFP acts as a diagnostic marker for the identification of primary tumors. Therapeutic Targeting of the above said markers will again become sensitive and may cause a relapse. Even though performing radical surgery, LCSC's will not be killed completely. In those LCSC's, they may have a chance of higher AFP levels with blood vessel metastasis of several degrees of variations. They may again progress to middle or advanced stages. On verifying the EpCAM+ levels which will be at higher end can be considered as a target for controlling tumor progression [49]. The regulatory maintenance of these marker initiations has been changed to RAS whereas it has been usually done in normal stem cells through Wnt, TGF-β, Notch, Hedgehog, Myc, and Bmi1. Hence, it must be necessary for the development of conditional therapeutic targets to suppress recurrence.

Bladder cancer: Urothelial cancers can also be named as Bladder cancer is the ninth most common cancer in the world. Nearly, 4 million new cases were diagnosed at which men were more affected than women. In continents like Africa and the Middle East, we can observe this type of cancer is due to smoking, higher BMI or infested water followed by higher incidence in developed countries. Tumor microenvironment will be enervated due to genotype heterogeneity and epigenetic alterations. It is necessary to know the stemness that shows the response towards preapoptotic signals, controllable signaling between proliferation and differentiation. Urothelial, basal and interstitial stratum cells are playing a pivotal role in the maintenance of stemness at which each cell has its derivation of either muscular or non-muscular and invasive or non-invasive bladder cancers [50-52]. Phenotypically, BCSC's are synthesized by three possible pathways bringing mutations in self-renewal capacity. Turning gene mutations in progenitor stem cells and differentiated to dedifferentiated cells are the primary targets for the synthesis of BCSC's [53, 54].

Regulating signaling pathways other than self-renewal and dedifferentiation, Epithelial Mesenchymal Transition (EMT) is an important pathway to convert normal stem cells into CSC's. This transitional cell carcinoma can be identified by specific phenotypic cell surface markers like CD 44, a primary marker identified by Li et al. [55]. Other remarkable features to identify the BCSC's are 67 LR+, CEACAM, and ALDH1 phenotypic markers involved in the cell adhesion and self-renewal properties for the maintenance of the plasticity. These marker regulators regulate through Hedgehog, Notch and Wnt to maintain stemness for tumor progression display poor therapeutic progress.

Pancreatic cancer: Difficulty in early prognosis is the primary therapeutic lock that can be identified only in the advanced stage of pancreatic cancer which was the 12th most common cancer in the world conjoined kidney cancer. On observation, Globocan reports state that nearly 3 million new cases have been diagnosed by 2012 at which the highest incidence can be seen in Czech Republic which is more commonly seen in developed countries and fatal diseases. Pancreatic cancer occupies the ninth position in Western Europe which is seven times higher in comparison with middle Africa. This distressing statistic will make us frighten that by 2030 it will become second leading cancer after Non-small lung cancer [56]. Higher incidence of pancreatic adenocarcinoma can be seen in men than in women on aging and predomination shows specificity towards ethnicity [57]. Contributing characteristics for procuring these cancers are primarily due to factors like environmental (smoking, alcohol, tobacco chewing) and genetic (Diabetes, Obesity, and pancreatitis).

Plasticity elucidation makes our concentration towards the stem cell nature of the pancreas. The stable unidirectional differentiation process can be termed as the Plasticity of the cell. Acinar cells of the pancreas may exhibit plasticity by undergoing EMT transdifferentiation. Desmoplastic has been closely associated with the pancreatic adenocarcinoma niche which accepts the bridge synonyms like Tumor microenvironment, crosstalk between CSC's and TME, EMT and CSC stemness/ EMT and non-CSC associated with tumor relapse [58].

Present studies are more focused on understanding inter/ intratumor biology metastatic cells which are deprived of nutrition, oxygen and delineation of a pattern of symmetrical division become chemotherapeutic resistant. To reduce these external pressures, the system develops a mechanism Autophagy by reducing the number of cells through engulfing. Effected performance of autophagy in these PDAC's/ PACSC's are due to epigenetic aberrations in the mediators Beclin1, Atg3 and conversion of the LC3B-I protein to LC3B-II might be one of

the reason for poor prognosis [59-61]. Characteristic tissue-specific EMT and functional EMT studies through dissection give insights about its redundancy.

Even though still under debate, it makes us turn our focus on understanding the basic evolutionary PDAC's. One of the mechanisms of either classical or self-renewal dedifferentiation will take place in response to the external signals received by cell surface markers like LGR5, EpCam, CD44, CD90, CD24, CD133, CXCR4 and aldehyde dehydrogenase1 (ALDH1). Marker operations will be carried out through JAK/STAT, Hedgehog, Wnt/s-catenin, and PI3K/AKT signaling [58, 62].

DISCUSSION

Normal cells of tissue is purely based on its characteristic features like growth, the balance between metabolic and efflux and influx, response towards apoptotic signals, normal cellular progeny synthesis with cell adhesion boundaries and so on. Whenever the breakage of connecting links between cellular phenotype and genotype occurs, it leads to abnormality in cellular growth. The abnormal phenomena will be termed as malignancy.

The tumor has a subset of multipotent, self-renewing cells called Cancer Stem Cells (CSCs) drive for tumor growth and relapse. CSC hypothesizes as under clonogenic division, development of cellular heterogeneity to persist against aggressive therapies by developing resistant stem cells. These CSC's are enriched with utilize multiple cell-surface marker strategies that include CD133, CD44, CD49f, CD36, ALDH, epidermal growth factor receptor (EGFR) and cytoskeletal markers like EPCAM, L1CAM, and others. The first CSC's identified in a childhood cancer found to express hematopoietic stem marker CD34. One of the important recognizable properties of CSC is that it resists too many therapeutic approaches like radiation and chemotherapy. These therapeutic approaches have increased efficacy towards non-stem tumor cells but do not effectively target CSCs which often enriches in treated tumors.

Present therapies induce stem cell state that generates stresses and shows the impact on tumor microenvironment. This brings alterations in pH, oxygen content, or nutrient supply. To target CSC Therapeutics, CSCs are frequently refractory to therapeutics that become resistant thereby attaining the ability to repropagate the tumor mass. Due to the production of toxic free radicals from water, it affects primarily DNA through mutations. Despite several adaptive mechanism alterations CSC niche survives become more clinical evaluation for the development of therapeutic inhibitors.

An interesting characteristic feature of CSC that makes an oncologist for non-effective therapy is self-renewal with asymmetrical targets on exposure to treatment becomes resistant. For self-renewal mechanism, CSC's are present with distinct anatomical niches which are not randomly distributed inside the tumor. These contain nutrients, oxygen, and physical and soluble interactions that maintain CSC self-renewal. The relationship between CSCs and their niches is dynamic thereby activates regulatory niche formation and maintenance. Studies on a hypoxic niche are not well defined structurally, characterized by low oxygen tension and increased acidity. Many studies have been conducted on multiple niches that include perivascular, proliferative, hypoxic and perinecrotic niches. Proximity to vascular endothelial cells has shown to regulate directly CSC growth. On ablation of the vasculature, it leads to tumor regression. Based on this, it makes us think about the identification of specific hallmark associated CSC in the tumor counteracting tumor hypoxic studies. These studies further will be required to understand the underlying mechanisms of resistant CSC's within tumor niche between Intra and inter tumors.

CONCLUSION

Hence, for the treatment of cancer, two strategically developments are necessary. One, general management of the patient should be performed in a multidisciplinary approach especially those who are having primary tumors needs surgical intervention. Second, more emphasis and deep studies about specific hallmark associated CSC induced hypoxia thereby identification of factors involved during tumor angiogenesis will break the stem cell plasticity and its therapeutic control.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

Data presented in this review article is a part of the Science and Engineering Research Board sponsored project from the Department of Science and Technology, Govt of India with file no. SB/FTP/ETA-0234/2013.

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