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Review Article

Cancer Stem Cells: Potential For Treatment

CANCER STEM CELLS

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Abstract: Cancer stem cells (CSCs) are unique cells within tumors that resist standard treatments and initiate tumor formation. Their presence and that of their progenitors contribute to tumor complexity, posing challenges to effective cancer therapies. Nevertheless, research on CSC biology holds promise for targeted therapies and reducing disease recurrence. This review provides a concise overview of CSCs and recent studies to enhance understanding their role in tumor heterogeneity and the tumor microenvironment, thereby advancing cancer research. CSCs exist in various cancers and can self-renew and differentiate into multiple cell types within tumors. These properties make them primary drivers of tumor growth and progression. Importantly, CSCs exhibit inherent resistance to conventional treatments like chemotherapy and radiation, making them formidable obstacles to successful outcomes. Recent studies have shed light on the intricate biology of CSCs, uncovering vulnerabilities and potential targets for novel therapeutic approaches; by specifically targeting CSCs, treatment resistance may be overcome, eliminating cells responsible for tumor initiation and recurrence. The tumor microenvironment, comprising cellular and non-cellular components, is critical in supporting CSCs and promoting tumor growth. CSCs interact with stromal cells, immune cells, and the extracellular matrix, forming a complex network that fosters tumor progression and therapy resistance. Investigating these dynamic interactions is essential for identifying therapeutic targets and interventions that disrupt the supportive environment surrounding CSCs.In conclusion, CSCs present challenges due to treatment resistance and their role in tumor growth. However, ongoing CSC-focused research offers hope for targeted therapies and strategies to prevent disease recurrence. Understanding tumor heterogeneity and the interactions between CSCs and the tumor microenvironment is crucial for advancing cancer research and improving patient outcomes. Unraveling the complexities of CSC biology paves the way for innovative approaches to combat cancer at its core.

Keywords: CSC, EMT, Cancer, stem cells, metabolism, microenvironment

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1. INTRODUCTION

Cancer stem cells (CSCs), initially discovered in acute myeloid leukemia (AML) by Bonnet and Dick in 1997, share similar properties with normal stem cells, including the ability to self-renew and differentiate into different cell types[1]. This groundbreaking study revealed that AML CSCs, characterized by the CD34+ CD38- phenotype, constitute a small fraction (approximately 0.1-1%) of the tumor population but can generate AML when transplanted into mice. Subsequent research has confirmed the pivotal role of CSCs in promoting resistance to cancer therapies, such as chemotherapy and radiation, and enhancing metastatic potential, posing significant challenges in cancer management[2]. To identify and characterize CSCs, various markers have been identified that distinguish them based on their stem-cell-like properties within tumor populations. Some markers exhibit specificity towards particular cancer types, while others provide a broader representation of tumor-initiating cells. Accordingly, it is crucial to identify CSCs using specific markers tailored to the stage and type of cancer for effective treatment strategies[3]. One notable phenomenon linked to CSCs is the Epithelial-Mesenchymal Transition (EMT), which entails the loss of epithelial characteristics and the acquisition of mesenchymal traits by cancer cells.[4] This transition significantly enhances the invasive capacity of cancer cells and contributes to the progression of the disease. CSCs and their unique properties present a substantial challenge in cancer treatment. Their resistance to conventional therapies and their ability to drive tumor growth and metastasis underscores the importance of developing targeted approaches to eradicate CSCs. Furthermore, understanding the mechanisms underlying CSC-mediated resistance and metastatic potential is essential for developing novel therapeutic strategies. Thus CSCs, resembling normal stem cells, play a crucial role in tumor initiation, therapeutic resistance, and metastasis. Identifying specific markers for CSCs according to the tumor stage and type is crucial for effective treatment. The EMT process further enhances the invasive behavior of cancer cells. Advancing our understanding of CSC biology and developing targeted therapies hold promise for improved cancer management by tackling the underlying mechanisms associated with CSC-mediated resistance and metastasis. Despite recent advancements in cancer treatment, the results have not met the desired expectations. One of the significant breakthroughs in stem cell biology is the discovery of tumor-initiating cells (TICs) or cancer stem cells (CSCs)[5]. Through a well-known mechanism, certain tumor cells undergo mutations and epigenetic changes in their signaling pathways, leading to the development of CSCs. Evidence indicates that CSCs are crucial in tumor invasion, cancer recurrence, and resistance to chemotherapy and radiation therapies[6]. Despite multiple therapeutic methods, effectively targeting these CSCs still needs to be seen. Targeting can generally be achieved either directly or by disrupting the tumor microenvironment. Cancer stem cells (CSCs) constitute a minority fraction of cancer cells in a tumor and exhibit distinctive traits akin to stem cells. These CSCs are thought to arise due to genetic alterations impacting adult stem cells, which play a vital role in the development of organs and maintenance of tissue equilibrium[7].

2. PROFILING OF CANCER STEM CELLS

The identification of cancer stem cells (CSCs) began with the discovery of CSCs in acute myeloid leukemia, where they were found to have CD34+ CD38- characteristics similar to normal hematopoietic stem cells[8]. Subsequently, their tumor-initiating capacity was confirmed through transplantation into immune-deficient mice[9]. CSCs are typically defined by specific proteins or glycoproteins' presence, absence, upregulation, or downregulation. Given the specificity challenge in detection, employing multiple markers for CSC identification is essential. Recently, researchers have been using common cell surface markers to indicate the presence of CSCs in tumors. In 2003, breast cancer stem cells (BCSCs) were identified based on the expression levels of CD44+ and CD24- cell surface antigens, which are associated with increased invasiveness, migration, and proliferation. CD44, a transmembrane glycoprotein, plays a critical role in signaling by interacting with cytoskeletal proteins or regulating gene expression to modulate cell behavior. Presently, the most popular method for identifying CSCs is examining the expression of cell surface antigens. Consequently, researchers are actively working to identify specific markers that can accurately identify CSCs.

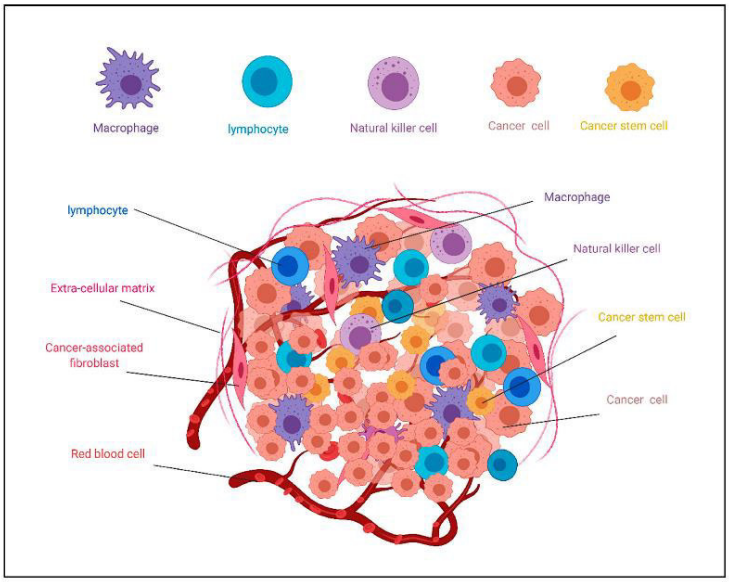

Fig 1: The tumour microenvironment (TME)'s heterogenicity is illustrated schematically[10].

3. IMPACT OF CSCS ON TUMOR VARIATION (HETEROGENEITY)

Intra-tumor heterogeneity is a complex phenomenon that emerges within tumors and their metastases, where cancer cells exhibit a wide range of genotypes and phenotypes. Various factors influence this diversity, including the uneven distribution of oxygen and nutrients within the tumor microenvironment. Additionally, aberrations caused by post-translational modifications and epigenetic changes further contribute to intra-tumor heterogeneity.[11] These alterations can result in distinct molecular profiles and functional characteristics among different cancer cell populations within the same tumor. The presence of intra-tumor heterogeneity poses a significant challenge for effective anticancer therapies[12]. The heterogeneous nature of tumors makes it difficult to adequately target all cancer cell populations. Moreover, some subpopulations of cancer cells may acquire resistance to treatment, leading to treatment failure and disease relapse. The dynamic and adaptive nature of intra-tumor heterogeneity necessitates a comprehensive understanding of its underlying mechanisms to develop strategies to address the diverse cancer cell populations within a tumor effectively.[13] Researchers employ DNA barcoding techniques to gain insights into specific cells' behavior and proliferative capacity within polyclonal populations. DNA barcoding involves labeling individual cells with unique, short DNA sequences, allowing for their identification and tracking within complex cellular populations[14]. This method enables the study of clonal expansion, cell fate determination, and lineage tracing in heterogeneous tumor environments. By analyzing these barcoded cells' proliferative capacity and behavior, researchers can gain valuable information about tumor growth dynamics, the hierarchical organization of cancer cell populations, and the influence of intra-tumor heterogeneity on therapeutic responses. In summary, intra-tumor heterogeneity arises from cancer cells with diverse genotypes, phenotypes, and access to oxygen and nutrients within tumors and their metastases. Aberrations resulting from post-translational modifications and epigenetic changes contribute to the complexity of intra-tumor heterogeneity, posing a significant challenge for effective anticancer therapies. DNA barcoding techniques provide a powerful tool for studying specific cell populations within heterogeneous tumor environments, enabling a better understanding of their proliferative capacity and behavior. Expanding our knowledge of intra-tumor heterogeneity and developing strategies to target its diverse cell populations are essential for advancing personalized and effective cancer treatments.

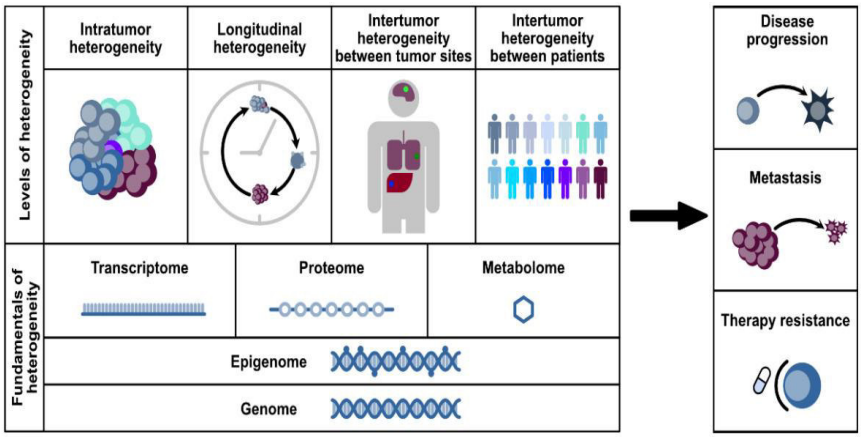

Fig 2: Heterogeneity levels. Different pathways found in the genome, epigenome, transcriptome, proteome, or metabolome contribute to the heterogeneity of tumours[15].

4. INFLUENCE OF CSCS ON EMT

The process known as Epithelial-Mesenchymal Transition (EMT) alters the characteristics of epithelial cell lines, causing them to acquire traits similar to mesenchymal cells during development [16]. EMT activation plays a crucial role in embryonic development and various processes, such as stem cell formation, wound healing, and the progression of carcinomas. This activation enables cells to gain mobility and invasiveness[17]. Throughout the progression of EMT, epithelial cells lose their adhesive properties, including tight junctions, desmosomes, and hemi desmosomes, resulting in a loss of apical-basal polarity (10, 29)[18]. The molecular mechanisms behind EMT can be classified into three groups: Inducers, regulators, and effectors. Inducers initiate the transition process when the tumor experiences hypoxia and nutrient deficiency as it grows. In carcinoma cells, EMT is characterized as "Type 3 EMT," which differs from developmental EMT, as oncogenes and tumor suppressor genes[19] greatly influences.

5. EPIGENETIC CONTROL OF CSCS

Cancer stem cells (CSCs) are a subpopulation with distinct chromatin features critical to gene expression regulation and chromatin architecture modification[20]. Among these features, the instability in chromosome length is a prominent characteristic often associated with overexpression of telomerase activity. This phenomenon has sparked a hypothesis that telomere repeats have a profound connection to the self-renewal of cancer stem cells, with telomerase activity as a vital factor in this process[21]. Recent research has focused on unraveling the intricate relationship between telomerase activity and CSCs. By understanding the mechanisms underlying these interactions, scientists hope to develop targeted medicines to disrupt cancer stem cell self-renewal. Such advancements could substantially impact combating cancer's regeneration capacity and ultimately improve the effectiveness of cancer treatments. A better comprehension of CSCs' chromatin features and the role of

telomerase activity could pave the way for novel therapeutic strategies, potentially leading to more personalized and effective treatments for cancer patients. Moreover, it could shed light on the elusive nature of cancer recurrence and metastasis, which are major challenges in cancer treatment. In conclusion, delving into the biology of cancer stem cells and their chromatin characteristics offers promising prospects for advancing cancer research and treatment. Exploring telomerase activity's involvement in CSC self-renewal may bring us one step closer to a future where cancer can be more effectively managed and, ultimately, defeated.

6. CORRELATION OF CSCS WITH EPIGENETIC MODULATION

Without changing DNA sequences, epigenetics is the chemical and physical alteration of chromatin and DNA that controls the expression of genes. Through DNA methylation, histone modifications, and non-coding RNAs (ncRNAs), which alter the accessibility of the chromatin or alter the expression of certain genes, epigenetics mediate gene expression [22]. Because epigenetic alterations are triggered by a person's genetic makeup or environmental events, they can potentially affect the development of pathological disorders, such as cancer. Because of this, harmful epigenetic changes can initiate or advance cancer [23,24] by acting as a cause, mediator, or effect of genomic instability. Like a DNA mutation, the underlying epigenetic signature in cancer cells is also known as "epimutation" and can cause uncontrollable cell proliferation. Without changing DNA sequences, epigenetics is the chemical and physical alteration of chromatin and DNA that controls the expression of genes. Growth-inhibiting signals, apoptosis, immortalization, angiogenesis, invasion, and metastasis are only a few of the resistance mechanisms mediated by epigenetics. Cancer stem cells (CSCs) exhibit distinctive chromatin properties that modulate gene expression by altering the structure of chromatin[25]. These cells possess unique characteristics, and their chromosomes often lack stability, possibly resulting from excessive telomerase activity. Telomeres, the repetitive DNA sequences located at the ends of chromosomes, are believed to play a crucial role in the self-renewal ability of CSCs[26]. The maintenance of telomeres is tightly controlled by the activity of telomerase, an enzyme responsible for adding telomere repeats to prevent chromosome shortening. Understanding the relationship between chromatin properties, telomerase activity, and CSC behavior could provide valuable insights into the development and treatment of cancer. The core characteristics of typical stem cells encompass regulatory systems and the ability to undergo symmetric (self-renewal) and asymmetric (pluripotency) division. These attributes are shared by Cancer Stem Cells (CSCs). However, CSCs possess distinct features that make them attractive targets for developing innovative treatment strategies that specifically target them (represented by the red arrows in the diagram). In the realm of cancer research, understanding the development of CSCs and tumor heterogeneity is of paramount importance. Three main processes are believed to contribute to the emergence of CSCs and tumor heterogeneity: Firstly, the dedifferentiation of mature cells, where specialized cells lose their distinct characteristics and revert to a less specialized state, potentially becoming CSCs. Secondly, differentiation arrest of adult tissue stem cells and/or progenitor cells leads to the accumulation of partially differentiated cells with stem-like properties. Lastly, trans differentiation of a stem cell from a different tissue, for instance, stem cells from the bone marrow migrating to other tissues and contributing to tumor formation. To explore the potential causes of CSCs in greater depth, researchers often refer to the most recent reviews (e.g., [14], [15]), which provide comprehensive explanations and insights into this complex phenomenon. Uncovering the underlying mechanisms behind CSCs can pave the way for novel and more effective therapeutic interventions in the ongoing fight against cancer[27].

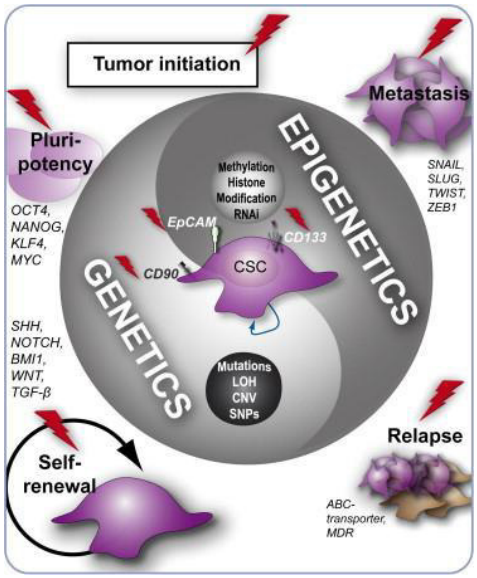

Fig 3: Tumor initiation: A circular diagram of the process[28]

7. CANCER INDUCTION BY STEM CELLS

Primitive tissue stem cells possess a unique characteristic that makes them prone to developing cancer-initiating mutations. These stem cells can self-renew and persist for extended periods, creating an environment where mutations can accumulate over time. Consequently, the accumulation of certain mutations could confer these cells with the capability to self-renew continuously, leading to the onset of transformative processes even in early progenitor cells (Tan

et al., 2006)[29]. The revelation of cancerous stem cells in leukemia sparked a wave of research to identify populations with similar properties in solid tumors. Almost a decade later, scientists made significant breakthroughs, discovering small populations of cells exhibiting tumor-initiating characteristics in mammary cancers (Al-Hajj et al., 2003)[30] and brain cancers. Interestingly, these brain cancer cells preferred forming tumors in mice with immunodeficiency (Singh et al., 2003, 2004)[31]. These findings have revolutionized our understanding of cancer development, emphasizing the significance of stem cells in tumor initiation and growth. Identifying these cancer-initiating cells provides potential targets for therapeutic strategies to eradicate cancer at its root and improve treatment outcomes. Continued research in this field holds promise for developing innovative therapies and advancing cancer treatment in the future. Stem cells, the cells with the longest lifespan in various tissues, are likely where the initial oncogenic mutations occur since multiple mutations are needed for cancer to develop. Researchers studying normal hematopoietic stem cells in leukemia patients who had been successfully treated discovered a significant AML1-ETO mutation. Genetic studies involving mouse models of brain, skin, and colon cancer showed that a single oncogenic mutation in either stem cells or their more differentiated offspring could lead to cancer 11, 31-35)[32-37]. The first oncogenic mutation(s) responsible for cancer often occurs in a stem cell. Still, the subsequent mutation that fully transforms the first cancer cell can arise in either a stem cell or an immature progenitor cell 22, 26, 37-41).[37-43]

8. CSCS AND PROGNOSTIC OUTCOMES

Cancer stem cells can be found in various types of myeloid leukemias and solid tumors such as glioblastoma, breast cancer, colon cancer, and skin squamous-cell cancers51[44]. The initial identification of cancer stem cells in solid tumors was accomplished by studying samples of human breast cancer cells that were transplanted into mice lacking a functional immune system52[45]. Cancer stem cells arising from a precursor cell rely on activating normal stem-cell pathways [46,47] and suppressing pathways that hinder stem-cell self-renewal. For long-term proliferation and invasion, cancer cells utilize the same self-renewal mechanisms as normal stem cells for tissue repair and regeneration[48], indicating a potential correlation between the frequency of stem cells in cancer and its prognosis. Both normal stem cells and their cancer stem-cell counterparts employ multiple mechanisms to safeguard themselves against toxins and genotoxic stress, such as increased expression of DNA-repair pathways, antiapoptotic gene activation, and the maintenance of a low level of reactive oxygen species (ROS) environment [49-51]. Several therapeutic agents, including certain chemotherapy drugs and radiation, induce cell death by elevating ROS levels.

9. DIFFERENTIATED TISSUES

Genomic instability is the basic driving force for cellular transformation, which begins in various cell types, including stem cells, progenitor cells, and mature cells. This important phenomenon involves the presence of aneuploidy and point mutations affecting proto-oncogenes or tumor-suppressor genes, respectively, at both the chromosomal and molecular levels (Lagasse, 2008)[52]. Numerous micro environmental elements actively contribute to the differentiation and transformation of cancer stem cells (CSCs) in the complex web of cancer development, which in turn aids in tumorigenesis. Notably, specific environmental conditions have the power to cause large genetic changes. For instance, radiation therapy, tissue damage, inflammatory cytokines like interleukin-6 (IL-6), and exposure to poisons can all result in mutations. Several genes, including the well-known tumor-suppressor gene p53, have mutations in them[53].Cancer can take advantage of weaknesses in the cellular architecture by disrupting genetic stability, which enables the conversion of healthy cells into malignant ones. The occurrence of genomic instability at the molecular and chromosomal levels is crucial for developing cancer's telltale symptoms, including unchecked cell proliferation, cell death resistance, and the capacity to infiltrate neighboring tissues[54].To create efficient treatment approaches, it is crucial to comprehend the complex link between genetic instability and the onset of cancer. It may be able to intervene and stop the development of cancer at its early stages by focusing on the underlying processes that cause genomic instability. Moreover, identifying and removing the microenvironmental elements that cause CSC differentiation and transformation can provide important insights toward properly avoiding and dealing with cancer.

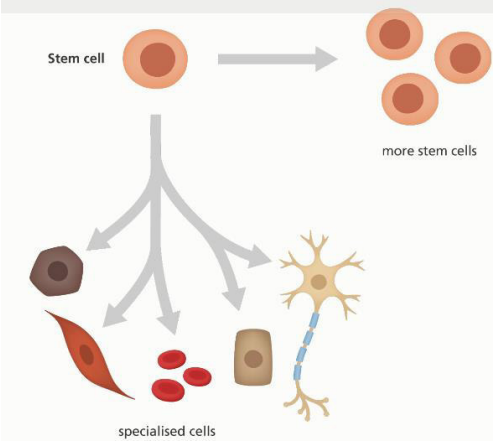

Fig 4: An embryonic cell producing more stem cells or specialized cells[55].

10. CELL HYBRIDIZATION (FUSION)

Cell fusion significantly influences both the beginning and development of cancer. The capacity to merge with many cell types, including hepatocytes, cardiac myocytes, oligodendrocytes, and Purkinje cells, is a property of migrating myeloid stem cells[56]. This fusion process can potentially trigger the development of cancer cells that can

spread throughout the body. Creating multinucleated cells known as heterokaryons, which result from fusing stem cells with adult cells, is a fascinating result of cell fusion (Duelli & Lazebnik, 2003; Pawelek, 2014).)[57,58]. These unusual cells, also known as cancer stem cells (CSCs), exhibit traits shared by differentiated cells and stem cells (Pomerantz & Blau, 2004).[59] This combination of features influences their involvement in the emergence of cancer. Tumors have a higher percentage of merged cells than other cancer cells, and these cells are more invasive and better able to migrate ((H. Li et al., 2014)[60]. The aggressiveness of tumours is aided by these fused cells, which make it easier for them to penetrate nearby tissues and spread to far-off locations inside the body. Learning how cells fuse in cancer offers important new insights into the processes causing tumour growth. Scientists can investigate cutting-edge tactics to interrupt or target cell fusion events by figuring out the mechanisms that underlie it, thereby limiting the ability of cancer cells to spread across the body. Furthermore, new therapeutic strategies may be created to selectively target and eradicate this particular population of cancer cells by concentrating on the distinguishing characteristics of CSCs resulting from cell fusion, thereby improving patient outcomes.

11. SUSTAINED PROLIFERATION

Cancer stem cells (CSCs) must be able to self-renew and asymmetrically produce new stem cells. This generates both stem and differentiated cells or symmetric (producing two similar stem cells) divisions (Van Pham,2015)[61] , enabling CSCs to persist indefinitely inside a tumour.Tumour serial transplanting is a method scientists use to demonstrate the self-renewal capacity of CSCs. In an early study, CSCs obtained from a tumour were transplanted into a mouse model to assess their tumorigenic potential during this experiment. Developing a new tumour after the transplant demonstrates the isolated CSCs' capacity for tumour growth and regenerative capacities(Huntly & Gilliland, 2005)[62].The regenerative behaviour of CSCs may be studied by tumour serial transplantation, which offers important insights intocrucial importance in the growth and formation of tumours. By using this approach, investigators hope to understand CSC behaviour better and pinpoint possible avenues for cancer therapy. Understanding the special characteristics of CSCs will enable the creation of new, more potent cancer-fighting strategies that will eventually enhance patient outcomes.

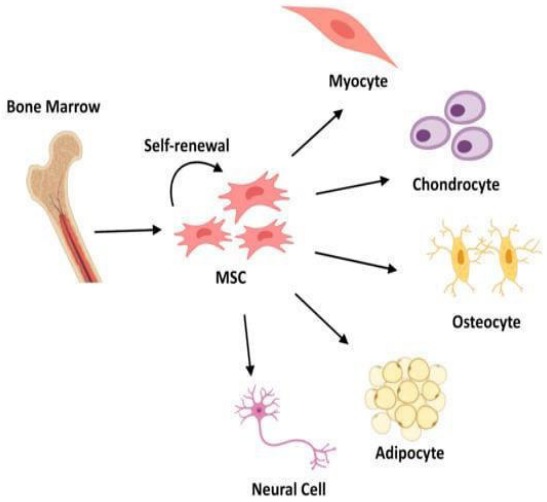

Fig 5: The development of mesenchymal stem cells (MSCs) into the three separate streams of myocytes, chondrocytes, osteoclasts, adipocytes, and neural cells is shown schematically[63,64].

12. PROGRAMMED CELL DEATH (APOPTOSIS)

The conventional focus of cancer therapy is on affecting tumour cell apoptosis. However, cancer stem cells (CSCs) have unique defenses that enable them to avoid apoptosis. As a result, researchers have looked at strategies to make CD133+ cells, a subset of CSC, more susceptible to the apoptosis brought on by hypoxia and serum depletion. A significant development in this direction came from Lin et al.'s 2012[65] work, which showed that preventing the heat shock protein Hsp27 from signalling was an effective sensitizing strategy. This discovery opens up a possible path for future therapeutic approaches that might target CSCs and improve the efficacy of cancer therapies. To overcome therapy resistance and provide new therapeutic options, researchers are working to understand and target the distinct survival processes of CSCs to higher success rates for cancer patients.By inhibiting Hsp27, CD133+ cells were made more vulnerable to apoptosis when starved of vital nutrients and exposed to low oxygen levels. The overproduction of the cellular signalling protein c-MET also plays a role in the resistance of CD133+ breast CSCs to apoptosis. This discovery, made public in 2012 by B. Sun, Liu, Xiao, and Zhu[66], emphasizes the intricacy of CSCs' strategies to avoid cell death. These insights present exciting opportunities for developing more specialized and potent cancer medicines that may more effectively combat the toughness of cancer stem cells, thereby improving the overall efficacy of cancer therapy approaches.

13. IMMUNOTHERAPEUTIC TARGETING OF CSCS[67]

There has been much study and investigation into new approaches to attack these hardy cells since cancer stem cells (CSCs) resist conventional cancer therapy procedures. Combination medicines, molecular inhibition, gene therapy, and immunotherapy have all shown promising results among these strategies. Targeting CSCs efficiently has a lot of potential, especially with immunotherapy. This ground-

breaking strategy uses the body's immune system to identify and eliminate cancer cells, including CSCs. Immunotherapy aims to improve the immune system's capacity to recognize and combat these elusive and resistant cancer stem cells by triggering the immune response. Contrarily, DNA therapy aims to change the behaviour of cancer cells and eventually trigger apoptosis by introducing particular genetic material into the cancer cells. By using the distinctive genetic traits of CSCs, this technique provides a more targeted and individualized approach to combating CSCs. Biological suppression aims to obstruct crucial signalling pathways and molecular targets necessary for the survival and expansion of CSCs. Scientists want to weaken the CSCs' resistance to traditional medicines and increase their susceptibility to therapy by sabotaging these vital systems. Additionally, the promise of combination treatments, which combine many forms of treatment at once, has been thoroughly studied in early studies[68]. This strategy tries to simultaneously target CSCs from many aspects, improving the chances of success and preventing the emergence of resistance. The goal is that as research into these alternative approaches develops, new avenues may open up for combating CSC resistance and enhancing patient outcomes from cancer treatment.

14. CANCER STEM CELLS AND RADIATION

Radiotherapy (RT) is still a practical and efficient method for treating different malignancies. A new age of three-dimensional conformal treatment has begun due to improvements in medical imaging and precise dose delivery methods, enabling more precise and targeted radiation therapy. Five radiobiological principles, generally called the "5 Rs in radiobiology," are essential to the efficiency of regular RT[69]. Repopulation, reoxygenation of hypoxic tumour areas, DNA damage repair, cell redistribution during the cell cycle, and radiosensitivity are some concepts[70]. Cancer stem cells (CSCs) differ from other cancer cells because they have unique traits that make them more radiation-resistant. Radiotherapy (RT) is still a practical and efficient treatment option for the tumour microenvironment-related extrinsic variables which impact CSCs' resistance to radiation. The lack of oxygen leads to hypoxia, which increases CSCs' radiation resistance. The capacity of CSCs to withstand radiation is also influenced by angiogenesis-related characteristics, such as the generation of vascular endothelial growth factor (Bao, Wu, Sathornsumetee et al., 2006))[71]. Even though radiation has significantly increased cancer patients' overall lifespan and quality of life, intrinsic variables remain serious obstacles that might cause relapse even after a patient has achieved full remission. To improve long-term results in cancer treatment, it is imperative to address the special radioresistant traits of CSCs. Current research emphasizes comprehending the complexities of CSCs and creating unique techniques to get around their radioresistant characteristics, bringing additional optimism for better cancer therapies in the future. This will increase the usefulness of radiation.

15. CSCS IN HYPOXIC NICHES AND TREATMENT RESISTANCE

Cancer stem cells (CSCs) from various tumour types are prone to developing treatment resistance, and hypoxia is a critical factor in this development. The activation of certain signalling pathways, such as the stemness and epithelial-to-mesenchymal transition (EMT) programs [72-76] results in the development of this resistance. CSCs become enriched for resistance to both chemotherapy and radiation when they are subjected to hypoxic stress using a variety of methods. Stimulating hypoxia-inducible factor (HIF) signaling, which results in the overexpression of stem-related pathways, including CD44 and Notch signaling, is one of the key processes. Studies carried out by Wu et al. in 2017 have provided information on these CSC adaptations to hypoxic circumstances[77].Due to their significantly reduced generation of reactive oxygen species (ROS)[78]CSCs also have a notable advantage over non-stem tumour cells in tolerating therapeutic stresses in hypoxic environments. In response to hypoxia, CSCs also exhibit the ability to migrate, which helps them avoid therapy and metastasis. This ability is aided by increased EMT signalling and changes in adhesion receptor expression[79-81]. To create targeted medicines that can successfully combat CSCs' resiliency and enhance overall treatment results for cancer patients, it is essential to understand the processes behind hypoxia-induced therapeutic resistance in CSCs.

16. CSCS AND IMMUNE RESPONSE

According to the 2018 study by Thorsson et al., immune cell infiltration inside solid tumours plays a complex and varied role, with various immunological components being co-opted to provide pro-tumorigenic roles.[82]. Different tumour forms differ in the makeup and frequency of immune cells. However, key interactions are common to many solid tumours. Cancer stem cells (CSCs) have developed ways to thwart the immune system's attempts to destroy them. These smart cells use a variety of tactics to evade being recognized by and eliminated by immune cells. One such strategy is the release of several immunosuppressive substances, such as TGF-b and IL4, which successfully reduce the anti-tumor immune response[83].CSCs produce an inhibitory microenvironment by secreting these immunosuppressive chemicals, which prevents immune cells from activating and functioning in a way that would normally allow them to recognize and fight the tumour. Because of their resilience and longevity inside the tumour, CSCs provide a difficult target for both immunotherapies and conventional cancer treatments due to their immune evasion. To create novel therapeutic strategies that overcome immunosuppression and bolster the immune response against CSCs[84]., ultimately enhance the efficacy of cancer treatments and enhance patient outcomes, it is essential to comprehend the complex interactions between CSCs and the immune system.

17. CSC-DIRECTED THERAPEUTIC APPROACHES

Specific cell markers that produce different tissue-specific fingerprints are used to identify and isolate cancer stem cells (CSCs). As shown in Table 1, some of these markers are often expressed in multiple cancer types, whereas others are restricted to particular organs. These indicators are useful for locating and investigating CSC communities because they reveal their variability and possible treatment weaknesses. Various treatment strategies have been used to effectively target CSCs, each specifically designed to meet the unique characteristics of these elusive cells. One such strategy includes directly inhibiting the self-renewal and tumorigenic potential of CSCs by targeting the particular cell surface markers that are overexpressed on CSCs[85].Also, medicines

that interfere with the molecular machinery and signalling pathways necessary for CSC survival and maintenance have been developed. Researchers hope to reduce CSC resistance to conventional therapies by focusing on these vital pathways. In addition, immunotherapeutic approaches have been investigated to use the immune system's capacity to identify and get rid of CSCs. Immunotherapy targets and eliminates these tenacious cells by eliciting an immune response against CSC-associated antigens. Combination treatments that combine several techniques have also been used to address CSC populations more effectively[86,87]. Combination medicines aim to have a more significant and long-lasting effect on tumor elimination by simultaneously targeting CSC-specific vulnerabilities and conventional cancer cells. These many therapeutic methods reflect the continual attempts to address the problems presented by CSCs and improve the efficacy of cancer therapies[88-91]. To develop more effective and individualized treatments for cancer patients, it is still crucial to comprehend the intricacy of CSC biology and its interactions with the tumor microenvironment.

Table 1: Accessing CSCs was done using methods that have undergone laboratory testing. Monoclonal antibody treatments and myeloid cell-based vaccinations constituted both of the major treatments.

Target	Tumor Type	Therapy	Clinical Trial/Reports	Results	Status
CD133	Advanced cholangiosarcoma	Cocktail CD133 CAR-T and CART-EGFR	NCT02541370 (Phase I and II)	Toxicity	Ongoing
CD133	Glioblastoma multiforme	ICT-121 DC vaccine	NCT02049489 (Phase I)	Not published	Ongoing
CD44	Refractory Acute Myeloid Leukemia	Monoclonal antibody (RG7356)	146 Limited clinical activity		
EpCAM	Metastatic colorectal cancer	Edrecolomab (Monoclonal antibody)	151 Limited response	150 Not statistically significant	
EpCAM	Metastatic colorectal cancer	3622W94 (Monoclonal antibody)	152 Toxicity		
EpCAM	Metastatic colorectal cancer	ING-1 (Monoclonal antibody)	Toxicity		
EpCAM	Metastatic colorectal cancer	Adecatumumab (Monoclonal antibody)	153 Favorable response in patients with the highest expression		
EpCAM	Various types of tumours	EPCAM-targeted CAR-T cells	NCT02729493 (N/A)	Ongoing	Ongoing
EpCAM	Various types of tumours	EPCAM-targeted CAR-T cells	NCT02725125 (N/A)	Ongoing	Ongoing
EpCAM	Various types of tumours	EPCAM-targeted CAR-T cells	NCT02915445 (Phase I)	Ongoing	Ongoing
EpCAM	Various types of tumours	EPCAM-targeted CAR-T cells	NCT03013712 (Phase I and II)	Ongoing	Ongoing
CD47	Various solid tumours and hematological malignancies	Hu5F9-G4 (Monoclonal antibody)	NCT02216409 (Phase I)	Not statistically significant	Ongoing
CD47	Various solid tumours and hematological malignancies	Hu5F9-G4 (Monoclonal antibody)	NCT02678338 (Phase I)	Not published	Ongoing
CD47	Various solid tumours and hematological malignancies	Combination of Hu5F9-G4 and Cetuximab	NCT02953782 (Phase I and II)	Ongoing	Ongoing
CD47	Various solid tumours and hematological malignancies	Combination Hu5F9-G4 and Retuximab	NCT02953509 (Phase I and II)	Ongoing	Ongoing
CD47	Various solid tumours and hematological malignancies	CC-90002 (Monoclonal antibody)	NCT02641002 (Phase I)	Ongoing	Ongoing
CD47	Various solid tumours and hematological malignancies	Combination CC-90002 and Retuximab	NCT02367196 (Phase I)	Ongoing	Ongoing
Autogenic glioma stem-like cells (A2B5+)	Glioblastoma multiforme	Dendritic cell-based vaccine	NCT01567202 (Phase II)	Ongoing	Ongoing

18. CONCLUSION

The specificity of the targeted antigens remains a hurdle despite advancements in developing CSC-targeting cancer treatments. Most treatments target indicators present in regular tissues and CSCs but aren't particularly induced by CSCs. Unfavourable effects include antigens poisoning levels rising as a result of treatment. Genomic, proteomic, and data mining techniques (Bioinformatics) applied to a large-scale analysis of tumour samples from various patients and cohorts may help identify CSC-specific mutations or allergen patterns that will enable the creation of more effective CSC-targeted therapies with fewer side effects for patients. Additionally, these methods could offer patient-specific signatures that permit individualized therapy and lower cost and toxicity. To target CSCs, new therapeutics, including gene targeting, nanoparticle-based treatments, and HDAC inhibitors, may be developed. In conclusion, the specificity of the targets is crucial for effectively eradicating CSCs and cancer recurrence, whether utilizing medicines, antibodies, or DNA therapy.

19. AUTHORS CONTRIBUTION STATEMENT

Dr. Somenath Ghosh conceived the study and was responsible for the overall direction, analysis, and planning. Durga Prasad TS, Ranjeet Kumar Chourasia Scarried out the implementation. Prof. Dr. Ammar A. Razzak Mahmood took the lead in writing the manuscript. Dr. Somenath Ghosh, Durga Prasad TS, Ranjeet Kumar Chourasia provided critical feedback, reviewed, and helped in the final corrections of the manuscript.

20. CONFLICT OF INTEREST

Conflict of interest declared none.

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