Global Journal of Medical Research: F
Diseases
Volume 23 Issue 2 Version 1.0 Year 2023
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Cancer Stem Cells as the Key to Cancer: Special Emphasis on Prostate Cancer

By Ghayeel Abo Kassm, Gaelle Antar, Maya Atwi, Tony Butrus, Elias Hajjar, Osamah Jaafar, Marita Machrekeki, Eddy Mikhael, Jessica Swesa, Fadi Mikhael & Muriel T. Zaatar

American University in Dubai

Abstract- Recent research into cancer stem cells has refined our knowledge of the origins, maintenance, and progression of cancer. The characteristics of tumor initiating cells and the stem-like properties of tumor side populations that appear to be responsible for tumor maintenance and metastasis have given insights into potential targets for the elimination of treatment-resistant and residual tumor cells. These insights have also provided inroads to understanding and preventing invasive and metastatic progression of cancer. In this review, we discuss recent advancements in understanding of tumor initiating cells and cancer stem cells and their implications on cancer pathobiology and treatment. The role of tumor initiating cell phenotypes on routes of metastasis and the use of stemness markers to guide prognosis and treatment are also discussed. Particular emphasis sections are included that focus on the role of stemness in the pathobiology and treatment of prostate cancer. Of particular interest is the correlation of stemness with decreased androgen receptor expression and resistance to anti-androgen therapy. The overview provided herein represents a primer for the understanding of current knowledge regarding cancer stem cells and their clinical implications in prostate and other cancer types.

GJMR-F Classification: DDC Code: 616.994061 LCC Code: RC271.C5

Cancer Stem Cells as the Key to Cancer: Special Emphasis on Prostate Cancer

Ghayeel Abo Kassm [α], Gaelle Antar [σ], Maya Atwi [ρ], Tony Butrus [ω], Elias Hajjar [¥], Osamah Jaafar [§], Marita Machrekeki [χ], Eddy Mikhael [ν], Jessica Swesa [Θ], Fadi Mikhael [ζ] & Muriel T. Zaatar [£]

Abstract- Recent research into cancer stem cells has refined our knowledge of the origins, maintenance, and progression of cancer. The characteristics of tumor initiating cells and the stem-like properties of tumor side populations that appear to be responsible for tumor maintenance and metastasis have given insights into potential targets for the elimination of treatment-resistant and residual tumor cells. These insights have also provided inroads to understanding and preventing invasive and metastatic progression of cancer. In this review, we discuss recent advancements in understanding of tumor initiating cells and cancer stem cells and their implications on cancer pathobiology and treatment. The role of tumor initiating cell phenotypes on routes of metastasis and the use of stemness markers to guide prognosis and treatment are also discussed. Particular emphasis sections are included that focus on the role of stemness in the pathobiology and treatment of prostate cancer. Of particular interest is the correlation of stemness with decreased androgen receptor expression and resistance to anti-androgen therapy. The overview provided herein represents a primer for the understanding of current knowledge regarding cancer stem cells and their clinical implications in prostate and other cancer types.

I. Introduction

Despite the significant advancements in cancer therapy throughout the years, cancer remains the most common cause of death worldwide [1]. Knowledge of how cancer initiates and the cellular and molecular origins of cancer continue to grow and be refined. Cancers have been thought to be monoclonal, meaning that each primary tumor originated from a single mutated cell. Mutation in one of a variety of genes may cause cells to form a tumor, while three to seven mutations and/or chromosomal defects may be needed for the development of cancer[2]. Accumulation of mutations can occur over time leading to cancer[2]. Complicating the monoclonal view of cancer, cancer growth and development are impacted by tumor heterogeneity and cell fusion. Recent research has shown that tumor cells and lymphocytes can merge, resulting in phenotypic and genotypic variation in tumor cells [3].

Author α σ ρ ω ¥ § χ ν Θ : Department of Biological and Physical Sciences, American University in Dubai, Dubai, United Arab Emirates.
Author ζ: Oncology Department, Mediclinic City Hospital, Dubai, United Arab Emirates.
Corresponding Author £: Department of Biological and Physical Sciences, American University in Dubai, Dubai, United Arab Emirates.
e-mail: mzaatar@aud.edu

A population of self-renewing cells with a high tumorigenic potential has been identified in many cancers, which are known as cancer stem cells (CSCs). The continuous and uncontrolled development of malignant tumors is thought to be caused by CSCs, which are also known as cancer-initiating cells (CICs)[2]. These cells are also thought to have a crucial role in metastasis and recurrence[2]. Many theories have suggested that the events occurring in either stem or differentiated cells, such as genomic instability, an inflammatory environment, genetic recombination, and lateral genetic transformation should be taken into consideration as potential CSC origins [2]. The ability of cancer cells to proliferate and, in many circumstances, survive is dependent on underlying stemness[4]. Moreover, due to cancer stem cells' capacity to trigger tumor growth, self-renewal, and multi-drug resistance, the majority of recent cancer research has focused on determining their distinctive characteristics and origins. CSCs have been identified in a variety of tumor types, including head and neck, stomach, breast, pancreatic, lung, liver, colon, melanoma, and bladder cancers[1].

Epithelial-to-mesenchymal transition (EMT) is a strictly controlled process that is essential for the development of tumors. EMT increases cancer cells' ability to migrate and invade and has a direct impact on the production of stem cell-like tumor-initiating cells. TGF-β1 plays crucial roles in the development of tumors and is a critical transcription factor regulating EMT [9]. Undoubtedly, all cells require energy for survival, proliferation, and cell growth. CSCs have a distinct metabolic flexibility in comparison to normal stem cells and significantly rely on oxidative phosphorylation (OXPHOS) as their main source of energy in contrast to non-CSCs, which are primarily glycolytic[5]. In the presence of oxygen, CSCs can alternate between OXPHOS and glycolysis to maintain homeostasis and consequently support tumor development [10].

The inner cell mass of the preimplantation blastocyst is a source of Embryonic Stem Cells (ESCs), which are distinguished and characterized by their pluripotency (the capacity and ability to differentiate into all derivatives of the three basic germ layers: ectoderm, endoderm, and mesoderm) and their potential to self-replicate without limit[6]. Apart from this, understanding originating cell types of cancer is a crucial step in determining mechanisms of tumor initiation and

maintenance. Long-term studies have related the development of prostate glands to stem cells. Prostate cancer is the second most prevalent cause of cancer-related death for men in the developed world , which is the most commonly diagnosed malignancy in males[7]. Regression of the prostate occurs following androgen deprivation, but regeneration occurs after testosterone replacement[8]. The cells responsible for this are located in the proximal ducts and basal layer of the prostate. Numerous characteristics of prostate cancer indicate a stem cell origin[8]. Surgery, radiation, hormonal ablation, and chemotherapy are examples of traditional anti-pancreas cancer treatments. For individuals with severe and/or metastatic cancer, these treatments are ineffective despite increased attempts. Nevertheless, cancer treatments frequently fail because of residual tumor cells that survive therapy, which causes the reappearance of the disease [7]. It has been suggested that CSCs represent this residual population. The general findings reported in the literature illustrate the connection between stem cells and prostate cancer, its therapies, the latest research on cancer stem cells, and potential future technologies to overcome it, which are discussed herein.

II. Stem Cells in Tumor Initiation, Tumor Cell Sustainability and Progression

As stated in Afify and Seno (2019), "Cancer stem cells (CSCs), also known as cancer-initiating cells (CIC), are responsible for the sustained and uncontrolled growth of malignant tumors and are proposed to play significant roles in metastasis and recurrence."[2] The authors clearly state that the initiation of cancer arises from stem cells. Furthermore, this statement is backed by research that was conducted by Mei et al. (2019), who presented very convincing evidence that CSCs have a substantial role in initiation of cancer[9]. This evidence shows that while there is a good understanding of how cancer cells form, the ability to prevent this from occurring remains elusive[10].

A plethora of research has been conducted that strongly supports the role that prostate cancer stem cells (PCSCs) play in the initiation of prostate cancer [9]. This drives the hypothesis that prostate stem cells are targets for prostate cancer initiation. Furthermore, it was proven by Eder et al. (2016) that cancer-associated fibroblasts (CAFs) and prostate cancer cells interact, which allows prostate cancer to proliferate and spread throughout the body [11]. Additional research by Begum et al. (2019) further supports this view [12]. They found that cancer-associated fibroblasts promoted CSC frequency, self-renewal, and metastasis in models of pancreatic ductal adenocarcinoma.

III. Inverse Correlation of Androgen Receptor Expression with Stemness in Prostate Cancer

Cancer progression is defined by continuous loss of a specific phenotype and the growth of progenitor and stem cell features[13]. In prostate cancer, androgen receptor (AR) signaling is important for the development of cancer and therapy resistance. AR signaling is decreased at the transcriptional level in high-grade versus low-grade prostate cancer. Resistance to androgen receptor therapy may be accompanied by loss of androgen receptor signaling and gain of stemness since loss of AR expression is associated with the development of stem cell-like features[13]. One way to inhibit AR signaling is by using the AR antagonist enzalutamide, which is one of the main treatments used for men with castration-resistant prostate cancer[14]. Furthermore, MDM2, an E3 ligase, allows for the ubiquitination of AR in CSCs, decreasing total AR protein levels[15]. The loss of MDM2 allows for the accumulation of AR leading to differentiation into luminal cells and cell death[15]. Blocking MDM2-mediated activity in concert with AR-targeted therapy can provide an approach for eliminating AR-negative CSCs in addition to AR-positive prostate cancer cells, which in turn decreases metastatic tumor burden and inhibits therapeutic resistance [15]. A study on the effects of AR demonstrated the influence of AR on the expression of CD44 and SOX2[16]. The experiment consisted of expressing AR in PC3 cells that are AR-negative. The expression levels of CD44 and SOX2 were decreased, indicating that AR-signaling can reduce stemness characteristics of these cells.

IV. Role of Stem Cells in Tumor Progression

Numerous studies have introduced discrete identities of cells that have stem cell-like features and experience shifts to adapt to a changing microenvironment as the disease progresses. A tumor's cell-of-origin determines its characteristics, such as metastasis, drug resistance, heterogeneity, and immortality[17]. A tumor that originated from cancer stem cells arising late in the life of tumors will have limited metastatic ability, a homogenous phenotype, and a restricted chemokine-receptor profile[17]. Conversely, buildup of mutations in early stem cells can produce tumors with increased rates of metastasis that are driven by a heterogeneous collection of chemokine receptors[17]. The aggressive nature of tumors is dependent on the processes of tissue formation and differentiation that are applied in the early embryonic stages. For example, ectoderm and endoderm-derived tumors metastasize through thelymphatics, while

mesenchyme-derived tumors metastasize by hematogenous spread[17].

CSCs exhibit high plasticity, meaning that they can change their phenotype and their appearance. These changes can be caused by chemotherapy, radiotherapeutics, senescence, and resulting changes in the tumor microenvironment (TME) [17]. Senescence can have anti-tumor effects but can also have negative effects, such as the promotion of cancer stemness, which can in turn increase plasticity, leading to tumor relapse or metastasis [17], [18]. Recent studies have indicated the importance and urgency of diagnostic screening of the TME prior to and during treatment since therapeutic efficacy and adverse effects of anti-cancer drugs can be affected by the TME [19].

In recent years, studies have provided more evidence that cancer stem cells play a pivotal role in the regulation of the TME and immunotherapeutic response in HCC patients. Recent construction of an HCC stemness subtype classifier may offer insights into the interaction between CSCs and the TME and may also be an approach for selecting immunotherapeutic responders in the future[20].

The JAK/STAT3 signaling pathway has a significant role in different types of cancers. Its activation increases metastatic and tumorigenic capability and chemoresistance in cancer by enhancing epithelial-mesenchymal transition EMT, which is related to stemness[21]. EMT is a critical regulator of cancer progression, regulating cancer spread, invasion, and survival[21]. Once activated,STAT3 enters the nucleus through importin-β1 and allows expression of genes that promote pathways that are critical for cancer survival[21].

V. Cancer Stem Cells and Prostate Cancer Survival

A comprehensive study by Tsudenomi et al (2019) concluded that there is no obvious link between CSCs and a patient's ability to survive; however, it is an integral part of establishing a prognosis [22]. Conversely, another study by Yi et al. (2020) effectively proved that prognosis and CSCs have a more direct correlation than previously discussed[23]. Specifically, an experiment was conducted by Li et al. (2020) that showed "that B7-H4 is a potential PCa [prostate cancer] stemness-associated biomarker to predict the prognosis of PCa."[24] This means that the B7-H4 gene is a stem cell-related gene, the overexpression of which can cause tumors to grow, thus establishing a link between CSCs and poor prognosis.

VI. Stem Cell Markers

Over the years, biomarkers have been gaining attention, especially because they are used in diagnosis, therapy, and prognosis, mostly in cancer patients. Cancer stems cells have been known to drive tumor initiation and relapse[25]. Cancer stem cells originate from either differentiated cells or adult tissue resident stem cells. Their importance in disease and development has led to investigation and discovery of stem cell biomarkers. In order to identify CSCs and distinguish them from non-CSC cancer cells, a variety of markers have been used. Common markers are CD133, CD44, IL-6R, and ALDH[26], [27]. These markers, which are predominantly expressed on stem-like cells, correlate with apoptosis resistance and tumor cell growth as they are prevalent on CSCs with enhanced cellular survival phenotypes[28].

Genomic stemness-regulating regions have been investigated for use as a marker for stemness, such as the ERG + 85 enhancer region for leukemia stem cells [29].The use of a reporter to sort an ERG + 85^{High} fraction of acute myelogenous leukemia cells showed the ability of this population to reconstitute the original tumor heterogeneity and was used to identify a 4-Hydroxyphenyl retinamide as an inhibitor of leukemia stem cells. This demonstrates the use of CSC markers to drive drug targeting[29].

VII. Effects of CSCs on Anti-cancer Therapy

a) Correlation of Stemness with Therapy Resistance

CSCs are more resistant to traditional therapies than other tumor cells and can adapt quickly to changes in the microenvironment. Radiotherapy, chemotherapy, or the cessation of treatment can trigger CSC resistance[30]. Tumorcell stemness has been associated with immune checkpoint inhibitor (ICI) resistance. A recent study used RNA sequencing to identify a pan-cancer signature corresponding to the stem.sig stemness-associated gene list that was predictive of ICI immunotherapy response[31]. Using CRISPR datasets, a list of genes involved in stemness whose knockout resulted in enhanced tumor immune response was generated. This evidence indicated that cancer stemness is associated with immunotherapy resistance and provided a genetic stemness profile that may potentially predict immunotherapy response[32].

VIII. Mechanisms of Drug Resistance in Cancer Stem Cells

The mechanisms that protect CSCs from chemotherapy or radiotherapy are an area of ongoing investigation. Recently, emphasis has centered on the role of the DNA damage response (DDR) in the development of tumors. It has been reported that cancer metastasis may be facilitated by an enhanced DDR that shields CSC and chemoresistant cells from the genotoxic pressure of chemotherapeutic medicines or radiation[33].

CSC populations are thought to drive chemoresistance and cancer relapse because of the capacity to self-renew and specialize into a variety of cancer cell lineages in response to chemotherapeutic drugs. Additionally, CSCs have the capacity enter a quiescent non-proliferative state, which supports their capacity to resist chemo- and radio-therapy[33]. Commonly used chemotherapy drugs induce apoptosis in dividing cells. Although effective cancer treatments kill most growing tumor cells, some CSCs survive because of decreased proliferation and chemoresistance and can initiate a relapse [25].

Special Emphasis: Implications of CSCs on Anti-Androgen Therapy Response

Male patients with castration-resistant prostate cancer (CRPC) have the option of treatment with the androgen receptor (AR) antagonist enzalutamide [14]. However, there area significant number of patients that do not respond to the treatment, and the causes behind this resistance are mostly unknown. Research by Alumkal et al. (2020) showed that those with enzalutamide resistance should be enrolled in clinical studies to collect tissue biopsies and apply medications to overcome resistance [14]. Menssouri et al. (2021) posited that AR resistance is related to multiple transcriptional processes that were previously active in pre-treatment samples[34]. O'Reilly et al. (2019) showed that CSCs and tumor relapse are connected on many levels. Also, hypoxic conditions that result from AR resistance cause a variety of signaling pathways to be activated, which elevates stem cell markers and promotes prostate CSC proliferation[35]. Thus, targeting hypoxic signaling pathways might prevent stem cell appearance and lessen resistance. Androgen deprivation therapy resistance has been found to be facilitated by increased expression of Fra1 and PTTG1, which is induced by STAT3 binding to their promoters[21]. Similarly, the stemness of glioblastoma cells is maintained when RTVP1 expression is promoted by the binding of both C/EBPβ and STAT3 to the RTVP-1 promoter, which is linked to poor clinical outcomes[21]. These findings open the door to a more thorough comprehension of the significance of CSC in castration-resistant prostate cancer and resistance to AR antagonism with enzalutamide[36].

Recently, cell plasticity has become a target for therapy in prostate cancer. Tumor cells may transform into a distinct subtypes in response to anticancer therapy, such as the neuroendocrine phenotype, which is linked to treatment failure [37]. Sánchez et al. (2020) proposed a new mechanism for the plasticity of prostate cancervia AMP protein kinase[37]. Prostate cancer cells showed signs of neuroendocrine morphology and expressed more neuroendocrine markers and neuron-specific enolase, which was correlated with increased expression of stem cell markers and resistance to AR [37]. In stem-like cells, overexpression of AMPK reduced the expression of stem markers and hypoxia-inducible factor (HIF-1). Also, docetaxel sensitivity was restored in stem-like AMPK-transfected cells [37].

IX. Stemness as a Therapeutic Target

a) Sensitizing cancer stem cells to cytotoxic therapy/radiation

One promising method for sensitizing breast cancer stem cells (BCSCs) to cytotoxic therapy is targeting the Fbxw7 gene, which maintains cell dormancy. Inhibition of Fbxw7 stimulates BCSCs to progress from the G0 quiescence phase can sensitize these CSCs to current therapies [38], [39]. The antirheumatic drug, sulfasalazine, has also shown to be effective in achieving therapy success by making CSCs more sensitive to radiation [38]. Targeting ATM signaling using an ATM inhibitor is able to resensitizeCD44+/CD24− BCSCs to radiation [38]. Similarly, inhibition of ATM/ATR signaling and downstream targets such as PARP1 and Wee1 increased the sensitivity of CSCs of multiple cancer types to chemotherapy and radiation [40]. Moreover, the promotion of BCSCs development by HIF-1α in hypoxic conditions can be targeted using ganetespib (a second-generation HSP90 and HIF-1α inhibitor)to sensitize BCSCs to chemotherapy in vivo and in vitro [38]. In addition, sequential treatment of patient-derived colorectal cancer xenografts with 5-fluorouracil (5-FU) or chemoradiotherapy (CRT) followed by evofosfamide (a hypoxia-activated prodrug) inhibited tumor growth and decreased the colorectal cancer initiating cell fraction [41]. Furthermore, Croker et al. showed that the inhibition of ALDH activity by using ALDH inhibitors, such as all-trans retinoic acid (ATRA) and diethylaminobenzaldehyde (DEAB), can make TNBC cells more sensitiveto chemotherapy and radiotherapy [42]. Similarly, silencing ALDH gene expression in ALDH-expressing ovarian CSCs reverses chemoresistance in these cells [43].

Another method for sensitizing CSCs is by targeting NOTCH signaling, which has been shown to sensitize patient-derived glioma stem cells to radiotherapy in vitro and to prevent xenograft formation [44]. In addition, inhibiting WNT/β-catenin pathway by using imatinib, a c-KIT/CD117 inhibitor, or anti-CD117 siRNA can reverse chemoresistance [45]–[48]. This was shown in pre-clinical models where the number of cancer stem cells decreased in squamous cell carcinoma and breast cancer xenografts, allowing therapeutic resistance to be overcome [48]–[50][. Nanotechnology has offered a novel way to target CSCs by enhancing local drug delivery. For example, PEGylated gold nanoparticles fused with anti-CD44 antibody greatly enhanced the targeting of breast and gastric cancer stem cells [51], [52]. In addition, using

carbon nanoparticle-mediated hyperthermia allows heating of cancer stem cells to overcome resistance by generating intense localized heat inside these cells which can reach temperatures above 50 ◦C [53]. Finally, Hh-activated CAF targeting in patient-derived xenografts using smoothened inhibitors (SMOi) can inhibit FGF signaling to suppress CSC populations and overcome chemoresistance [54].

b) Targeted therapy directed toward cancer stem cells

Disrupting CAF-CSC crosstalk is an attractive approach to targeting CSCs. Using Stattic, a STAT3 inhibitor, to block IL-6/IL-6R/STAT3 signaling can reduce stemness of BCSCs [55]. Additionally, the STAT3 antisense oligonucleotide AZD9150 exhibits antitumor tumor activity in refractory lymphoma and NSCLC clinical trials [54]. Further, CCL2-neutralizing antibodies and inhibitors of α- and γ-secretases that activate NOTCH have reduced stemness and stopped metastasis of breast cancer cells and glioblastoma cells in preclinical studies [57], [58]. Moreover, using AMD3100 (plerixafor) to block SDF-1/CXCR4 signaling greatly suppresses the CSC population in breast, colon and renal cancers[59]–[61]. However, these interventions have been relatively ineffective in patients with solid tumors [60]–[63]. On the other hand, using BKM120 or Ly294002 to block PI3K/AKT signaling can kill CSCs in can kill colon, prostate and breast cancers [66]–[69], and the PI3K inhibitors PX-866 [73], alpelisib [74], PQR309 [75] and pictilisib [76] were effective in patients with solid tumors [70]–[73].

LGK974, Wnt-C59, and cyclosporin A, which inhibit the WNT/β-catenin pathway are able to inhibit the proliferation of CSCs in different cancers [74]–[76]. It has also been shown that vismodegib, a Hedgehog inhibitor, inhibits proliferation and triggers apoptosis in breast, colon, and prostate cancers [77]–[79]. Sonidegib, another hedgehog inhibitor, has shown to inhibit CAF activation and reduce the CSC population in triple-negative breast cancer[54]. Another approach is targeting the metabolism of CSCs. One of the most studied strategies that targets metabolism is the use of compounds that block electron transport chain (ETC) complexes, which inhibits mitochondrial respiration[80]. Antidiabetic drugs such as metformin and phenformin can act as ETC inhibitors to impair oxidative phosphorylation in CSCs[80]. In addition, antibiotics like doxycycline, tigecycline and bedaquiline can target mitochondrial translation and biogenesis {cite}. A method for selective drug delivery in mitochondria can be adopted using chemotherapeutics and small drug-conjugated nanocarriers[80]. Targeting lipid metabolism is another pan-CSC strategy. Stearoyl-CoA desaturase 1 (SCD-1) inhibitors have shown to target properties of stemness in cancer models in vitro and in vivo[80]. Statins can also be used to inhibit cholesterol synthesis via the mevalonate pathway[80]. Lipid uptake can be targeted using strategies revolving around inhibition of the transporter CD36 either pharmacologically or using blocking antibodies [80].

Treatment with salinomycin-encapsulated lipid-PLGA nanoparticles conjugated with CD44 antibodies has resulted in improved cytotoxic effects on CD44+ prostate cancer initiating cells with enhanced suppression of tumorsphere formation [81]. Using drugs, antibodies, vaccines, and CAR-T cells to target transcription factors, intracellular signaling pathways such as Hedgehog, Notch, Wnt signaling, extracellular factors, CSC-associated surface markers, apoptotic pathways, and CSC-niche interactions presents several effective ways to target CSCs [39], [82]. Lv et al. showed that vitamin C uptake via sodium-dependent vitamin C transporter 2 (SVCT-2) induced apoptosis in liver cancer stem cells in vitro and in vivo experiments [83]. Furthermore, in a phase II trial, Brown et al. demonstrated that using Metformin as a treatment caused a major reduction in the CSC population, a change in DNA methylation of carcinoma-associated mesenchymal stem cells (CA-MSCs), and elimination of increased chemoresistance caused by CA-MSCs [84].

c) Directing immunotherapy to cancer stem cells

A small number of immunotherapyoptions to target CSCs exist to date and include adaptive T-cells, dendritic cell (DC)-based vaccines, and immune checkpoint inhibitors[85], [86].The discovery of ICIs dramatically changed the standard-of-care practice in oncology allowing for the targeting of tumor immunity. CSCs represent a unique subpopulation of tumor cells that initiate and perpetuate tumors. CSCs are recognized as acore cause of drug resistance, cancer relapse, invasion, and migration. CSC self-renewal and immune evasion can be driven by dysregulated FTO(Fat mass and obesity-associated protein)[87]. FTO has been reported to be upregulated in many tumors[87]. Targeting FTO helps to suppress tumor growth, potentiates immunotherapy, and attenuates drug resistance[87]. Inhibition of FTO can dramatically change immune response by suppressing expression of immune checkpoint genes[87]. It has been reported that two potent small-molecule FTO inhibitors exhibit strong anti-tumor effects in multiple types of cancers[87]. This study was conducted using samples from patients with newly diagnosed, after treatment, or relapsed leukemia. Through a series of screening and validation assays the authors discovered that the FTO inhibitors CS1 and CS2 displayed potent anti-leukemic effects in vitro by selectively suppressing FTO activity and signaling leading to the activation of apoptosis. The potent anti-tumor efficacy and minimal side effects of CS1 and CS2 observed in this study suggest a high potential for clinical application. In addition to hematopoietic malignancies, FTO has also been reported to play oncogenic roles in many types of

solid tumors (glioblastoma, breast cancer, and pancreatic cancer)[87]. This evidence confirms the broad therapeutic potential of immunotherapy targeting CSCs in various types of cancers, particularly FTO inhibitors.

d) Vaccination against CSCs

A growing body of evidence suggests that complete tumor eradication is impossible without effective elimination of cancer stem cells (CSCs). The resistant nature of CSCs makes conventional chemotherapy inefficient. For example, breast cancer stem cells (BCSC) activate molecular pathways that render them resistant to current therapies, such as the increased functionality of DNA-repair mechanisms, the overexpression of detoxifying enzymes, enhanced anti-oxidant capabilities, and resistance to apoptosis [85]. Therefore, targeted immunotherapy using vaccines may be a compelling option [27]. BCSCs possess several mechanisms to evade the immune response, thus use of vaccines for the treatment of chemoresistant breast cancer, perhaps in combination with ICIs, may be an attractive modality. Currently, there are two tumor vaccine options that are being studied: DC (dendritic cell)-based vaccines [86] and vaccines consisting of irradiated induced pluripotent stem cells (iPSC). Both are undergoing clinical trials but have not thus far been approved for targeted immunotherapy for cancer[89].

X. Therapeutic Markers

CSCs express immune resistance markers and exhibit specific immune characteristics in various cancers. This phenomenon can be exploited using immunotherapies to target CSCs [27].A literature review concluded that as a sub population of bulk tumors, CSCs resist conventional cancer therapies, escaping from antitumor immunity through lower expression of immune receptors [89]. This prompts a drive toward the development of smarter, CSC-targeted, therapeutic approaches using specific CSCs markers.

a) Markers for the use of immune checkpoint inhibitors

The development of ICIs marked a new era in anti-cancer therapy. This treatment modality has resulted in favorable responses and substantial improvement in survival in various cancer types. Therefore, increasing attention is being paid to the identification of predictive biomarkers for the efficacy of ICIs. Identifying predictive biomarkers can help to understand whether ICIs will be effective in tumor suppression. Such information can influence decision-making toward individualized anti-tumor immunotherapy and help to monitor drug efficacy and progression of the disease. PD-L1 immune checkpoint ligand has been shown to be expressed highly in CSCs, to contribute to the stemness of these cells, and to mediate immune evasion [89].

XI. Looking Forward

Tumors consist of heterogeneous cell populations. This heterogeneity plays key roles in regulating tumor initiation, metastasis, recurrence, and resistance to anti-tumor therapies [39]. Defining the regulatory mechanisms of heterogeneity is essential for targeting BCSCs and treating breast cancer [90]. A recent study outlines discoveries of novel regulators of BCSCs and their niches for BCSC heterogeneity[54]. In this study, hedgehog signaling in tumor cells led to the reprogramming of cancer-associated fibroblast to support a CSC phenotype that was resistant to chemotherapy. The authors highlight that using this new data allows for better prognosis and prediction of therapeutic efficacy, which may provide novel and more efficient treatment strategies[54].

Hypoxia is a common feature of tumors that presents opportunity for future therapies, developing because of the rapid growth of tumors, outpacing oxygen supply. Hypoxia is affected by blood flow, which is counteracted by formation of abnormal blood vessels ("neo-vessels") supplying the tumor. Tumor hypoxia is associated with the invasion, metastasis, tumor survival, suppression of anti-tumor immunity, and hampered therapeutic response. Several potential mechanisms may play a role in these phenotypes, including alteredgene expression, activation of oncogenes, inactivation of suppressor genes, genomic instability, and clonal selection [90]. A. Emami Nejad et al. studied the effects of hypoxia on tumor biology and possible strategies to manage the hypoxic tumor microenvironment [68]. The authors noted that hypoxia enhances the aggressiveness of tumors and creates a barrier to conventional cancer therapy, including radiotherapy, chemotherapy, and phototherapy, confirming that tumors that are hypoxic are associated with a poorer outcomes[90]. The role of hypoxic CSCs in tumor expansion and malignant progression favoring immune escape has been highlighted[54].

The effects of hypoxia on tumor cells are mediated by hypoxia inducible factors (HIFs). HIFs upregulate the expression of angiogenic factors, particularly VEGF in CSCs, and promote tumor angiogenesis [90]. HIF proteins are master regulators of oxygen homeostasis. Therefore targeting HIFs is an attractive strategy in the treatment of tumors. Several approaches have been identified for targeting hypoxia including, hypoxia-activated prodrugs (HAPs), specific targeting of HIFs, and targeting downstream HIF signaling pathways or critical pathways specific to hypoxic cells (such as mTOR and UPR) [90]. A. EmamiNejad et al. concluded that HIF stabilization in hypoxic tumor cells induces the expression of specific target genes encoding proteins that promote neo-angiogenesis (VEGF), metabolic changes, stemness and metastasis[90]. It has also been noted that effective

anti-angiogenic (VEGF) therapy may be achieved in combination with inhibitors of tumor hypoxic adaptation [54].

XII. Conclusion

In summary, stemness in tumor cells is an indicator of therapeutic resistance and prognosis. Refinement of the markers of stemness used to identify these cells and their phenotypes in cancers is leading to the ability to predict treatment responses and develop new approaches to the effective elimination of resistant tumor cell populations. Better understanding of the nature of cancer stem cells heightens our awareness of the appropriate application of emergent therapy modalities, such as immune checkpoint inhibitors, tumor vaccines, and hypoxia-targeted drugs. Improved understanding of tumor biology is not possible without the intimate understanding of the role of the cancer stem cell as a critical player in the initiation, maintenance, and progression of cancer.

References Références Referencias

1. A. K. Yadav and N. S. Desai, "Cancer Stem Cells: Acquisition, Characteristics, Therapeutic Implications, Targeting Strategies and Future Prospects," Stem Cell Rev. Rep., vol. 15, no. 3, pp. 331–355, Jun. 2019, doi: 10.1007/s12015-019-09887-2.
2. S. M. Afify and M. Seno, "Conversion of Stem Cells to Cancer Stem Cells: Undercurrent of Cancer Initiation," Cancers, vol. 11, no. 3, Art. no. 3, Mar. 2019, doi: 10.3390/cancers11030345.
3. M. R. Atashzar et al., "Cancer stem cells: A review from origin to therapeutic implications," J. Cell. Physiol., vol. 235, no. 2, pp. 790–803, 2020, doi: 10.1002/jcp.29044.
4. P. M. Aponte and A. Caicedo, "Stemness in Cancer: Stem Cells, Cancer Stem Cells, and Their Microenvironment," Stem Cells Int., vol. 2017, p. e5619472, Apr. 2017, doi: 10.1155/2017/5619472.
5. V. Snyder, T. C. Reed-Newman, L. Arnold, S. M. Thomas, and S. Anant, "Cancer Stem Cell Metabolism and Potential Therapeutic Targets," Front. Oncol., vol. 8, 2018, Accessed: Oct. 27, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fonc.2018.00203
6. Y. Atlasi, L. Looijenga, and R. Fodde, "Chapter Thirteen - Cancer Stem Cells, Pluripotency, and Cellular Heterogeneity: A WNTer Perspective," in Current Topics in Developmental Biology, vol. 107, M. Rendl, Ed. Academic Press, 2014, pp. 373–404. doi: 10.1016/B978-0-12-416022-4.00013-5.
7. R. Leão, C. Domingos, A. Figueiredo, R. Hamilton, U. Tabori, and P. Castelo-Branco, "Cancer Stem Cells in Prostate Cancer: Implications for Targeted Therapy," Urol. Int., vol. 99, no. 2, pp. 125–136, 2017, doi: 10.1159/000455160.
8. S.-M. Tu and S.-H. Lin, "Prostate Cancer Stem Cells," Clin. Genitourin. Cancer, vol. 10, no. 2, pp. 69–76, Jun. 2012, doi: 10.1016/j.clgc.2012.01.002.
9. W. Mei, X. Lin, A. Kapoor, Y. Gu, K. Zhao, and D. Tang, "The Contributions of Prostate Cancer Stem Cells in Prostate Cancer Initiation and Metastasis," Cancers, vol. 11, no. 4, Art. no. 4, Apr. 2019, doi: 10.3390/cancers11040434.
10. A. Vicente-Dueñas, J. Hauer, C. Cobaleda, A. Borkhardt, and I. Sánchez-García, "Epigenetic Priming in Cancer Initiation," Trends Cancer, vol. 4, no. 6, pp. 408–417, Jun. 2018, doi: 10.1016/j.trecan.2018.04.007.
11. D.-Y. Sun, J.-Q. Wu, Z.-H. He, M.-F. He, and H.-B. Sun, "Cancer-associated fibroblast regulate proliferation and migration of prostate cancer cells through TGF-β signaling pathway," Life Sci., vol. 235, p. 116791, Oct. 2019, doi: 10.1016/j.lfs.2019.116791.
12. A. Begum et al., "Direct Interactions With Cancer-Associated Fibroblasts Lead to Enhanced Pancreatic Cancer Stem Cell Function," Pancreas, vol. 48, no. 3, pp. 329–334, Mar. 2019, doi: 10.1097/MPA.0000000000001249.
13. X. Liu, W. (Jess) Li, I. Puzanov, D. W. Goodrich, G. Chatta, and D. G. Tang, "Prostate cancer as a dedifferentiated organ: androgen receptor, cancer stem cells, and cancer stemness," Essays Biochem., vol. 66, no. 4, pp. 291–303, Sep. 2022, doi: 10.1042/EBC20220003.
14. J. J. Alumkal et al., "Transcriptional profiling identifies an androgen receptor activity-low, stemness program associated with enzalutamide resistance," Proc. Natl. Acad. Sci., vol. 117, no. 22, pp. 12315–12323, Jun. 2020, doi: 10.1073/pnas.1922207117.
15. P. Vummidi Giridhar, K. Williams, A. P. VonHandorf, P. L. Deford, and S. Kasper, "Constant Degradation of the Androgen Receptor by MDM2 Conserves Prostate Cancer Stem Cell Integrity," Cancer Res., vol. 79, no. 6, pp. 1124–1137, Mar. 2019, doi: 10.1158/0008-5472.CAN-18-1753.
16. A. Srinivasan, L. Senbanjo, S. Majumdar, R. B. Franklin, and M. A. Chellaiah, "Androgen receptor expression reduces stemness characteristics of prostate cancer cells (PC3) by repression of CD44 and SOX2," J. Cell. Biochem., vol. 120, no. 2, pp. 2413–2428, 2019, doi: 10.1002/jcb.27573.
17. V. Richard, T. R. S. Kumar, and R. M. Pillai, "Transitional dynamics of cancer stem cells in invasion and metastasis," Transl. Oncol., vol. 14, no. 1, p. 100909, Jan. 2021, doi: 10.1016/ j.tranon.2020.100909.
18. S. Qin, B. A. Schulte, and G. Y. Wang, "Role of senescence induction in cancer treatment," World J.

Clin. Oncol., vol. 9, no. 8, pp. 180–187, Dec. 2018, doi: 10.5306/wjco.v9.i8.180.
19. L. Walcher et al., "Cancer Stem Cells—Origins and Biomarkers: Perspectives for Targeted Personalized Therapies," Front. Immunol., vol. 11, 2020, Accessed: Nov. 03, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fimmu.2020.01280
20. A. Chen et al., "Integrated Machine Learning and Bioinformatic Analyses Constructed a Novel Stemness-Related Classifier to Predict Prognosis and Immunotherapy Responses for Hepatocellular Carcinoma Patients," Int. J. Biol. Sci., vol. 18, no. 1, pp. 360–373, Jan. 2022, doi: 10.7150/ijbs.66913.
21. W. Jin, "Role of JAK/STAT3 Signaling in the Regulation of Metastasis, the Transition of Cancer Stem Cells, and Chemoresistance of Cancer by Epithelial–Mesenchymal Transition," Cells, vol. 9, no. 1, Art. no. 1, Jan. 2020, doi: 10.3390/cells9010217.
22. R. Tsunedomi, K. Yoshimura, N. Suzuki, S. Hazama, and H. Nagano, "Clinical implications of cancer stem cells in digestive cancers: acquisition of stemness and prognostic impact," Surg. Today, vol. 50, no. 12, pp. 1560–1577, Dec. 2020, doi: 10.1007/s00595-020-01968-x.
23. L. Yi et al., "Integrative stemness characteristics associated with prognosis and the immune microenvironment in esophageal cancer," Pharmacol. Res., vol. 161, p. 105144, Nov. 2020, doi: 10.1016/j.phrs.2020.105144.
24. H. Li, L. Piao, S. Liu, Y. Cui, and Y. Xuan, "B7-H4 is a potential prognostic biomarker of prostate cancer," Exp. Mol. Pathol., vol. 114, p. 104406, Jun. 2020, doi: 10.1016/j.yexmp.2020.104406.
25. L. T. H. Phi et al., "Cancer Stem Cells (CSCs) in Drug Resistance and their Therapeutic Implications in Cancer Treatment," Stem Cells Int., vol. 2018, p. e5416923, Feb. 2018, doi: 10.1155/2018/5416923.
26. C. Wefers, G. Schreibelt, L. F. A. G. Massuger, I. J. M. de Vries, and R. Torensma, "Immune Curbing of Cancer Stem Cells by CTLs Directed to NANOG," Front. Immunol., vol. 9, 2018, Accessed: Nov. 04, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fimmu.2018.01412
27. N. Badrinath and S. Y. Yoo, "Recent Advances in Cancer Stem Cell-Targeted Immunotherapy," Cancers, vol. 11, no. 3, Art. no. 3, Mar. 2019, doi: 10.3390/cancers11030310.
28. Z. Wang, K. Zhao, T. Hackert, and M. Zöller, "CD44/CD44v6 a Reliable Companion in Cancer-Initiating Cell Maintenance and Tumor Progression," Front. Cell Dev. Biol., vol. 6, 2018, Accessed: Oct. 27, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fcell.2018.00097
29. M. Yassin et al., "A novel method for detecting the cellular stemness state in normal and leukemic human hematopoietic cells can predict disease outcome and drug sensitivity," Leukemia, vol. 33, no. 8, Art. no. 8, Aug. 2019, doi: 10.1038/s41375-019-0386-z.
30. M. Najafi, K. Mortezaee, and J. Majidpoor, "Cancer stem cell (CSC) resistance drivers," Life Sci., vol. 234, p. 116781, Oct. 2019, doi: 10.1016/j.lfs.2019.116781.
31. M. Zhang, X. Wang, X. Chen, F. Guo, and J. Hong, "Prognostic Value of a Stemness Index-Associated Signature in Primary Lower-Grade Glioma," Front. Genet., vol. 11, 2020, Accessed: Nov. 03, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fgene.2020.00441
32. "Integrated analysis of single-cell and bulk RNA sequencing data reveals a pan-cancer stemness signature predicting immunotherapy response | Springer Link." https://link.springer.com/article/10.1186/s13073-022-01050-w (accessed Nov. 04, 2022).
33. A. Abad, D. Graifer, and A. Lyakhovich, "DNA damage response and resistance of cancer stem cells," Cancer Lett., vol. 474, pp. 106–117, Apr. 2020, doi: 10.1016/j.canlet.2020.01.008.
34. N. Menssouri et al., "Abstract 358: A prospective study of prostate cancer metastases identifies an androgen receptor activity-low, stemness program associated with resistance to androgen receptor axis inhibitors and unveils mechanisms of clonal evolution," Cancer Res., vol. 81, no. 13_Supplement, p. 358, Jul. 2021, doi: 10.1158/1538-7445.AM2021-358.
35. D. O'Reilly, P. Johnson, and P. J. Buchanan, "Hypoxia induced cancer stem cell enrichment promotes resistance to androgen deprivation therapy in prostate cancer," Steroids, vol. 152, p. 108497, Dec. 2019, doi: 10.1016/j.steroids.2019.108497.
36. J. R. Federer-Gsponer et al., "Patterns of stemness-associated markers in the development of castration-resistant prostate cancer," The Prostate, vol. 80, no. 13, pp. 1108–1117, 2020, doi: 10.1002/pros.24039.
37. B. G. Sánchez, A. Bort, D. Vara-Ciruelos, and I. Díaz-Laviada, "Androgen Deprivation Induces Reprogramming of Prostate Cancer Cells to Stem-Like Cells," Cells, vol. 9, no. 6, Art. no. 6, Jun. 2020, doi: 10.3390/cells9061441.
38. S. Palomeras, S. Ruiz-Martínez, and T. Puig, "Targeting Breast Cancer Stem Cells to Overcome Treatment Resistance," Molecules, vol. 23, no. 9, Art. no. 9, Sep. 2018, doi: 10.3390/molecules23092193.
39. C. Saygin, D. Matei, R. Majeti, O. Reizes, and J. D. Lathia, "Targeting Cancer Stemness in the Clinic: From Hype to Hope," Cell Stem Cell, vol. 24, no. 1,

pp. 25–40, Jan. 2019, doi: 10.1016/j.stem.2018.11.017.
40. "Cancer stem cells: Road to therapeutic resistance and strategies to overcome resistance – Science Direct." https://www.sciencedirect.com/science/article/pii/S0925443918304769 (accessed Nov. 04, 2022).
41. J. Haynes et al., "Administration of Hypoxia-Activated Prodrug Evofosfamide after Conventional Adjuvant Therapy Enhances Therapeutic Outcome and Targets Cancer-Initiating Cells in Preclinical Models of Colorectal Cancer," Clin. Cancer Res., vol. 24, no. 9, pp. 2116–2127, Apr. 2018, doi: 10.1158/1078-0432.CCR-17-1715.
42. A. K. Croker and A. L. Allan, "Inhibition of aldehyde dehydrogenase (ALDH) activity reduces chemotherapy and radiation resistance of stem-like ALDHhiCD44+ human breast cancer cells," Breast Cancer Res. Treat., vol. 133, no. 1, pp. 75–87, May 2012, doi: 10.1007/s10549-011-1692-y.
43. B. N. Landen et al., "Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer," Mol. Cancer Ther., vol. 9, no. 12, pp. 3186–3199, Dec. 2010, doi: 10.1158/1535-7163.MCT-10-0563.
44. J. Wang et al., "Notch Promotes Radioresistance of Glioma Stem Cells," Stem Cells, vol. 28, no. 1, pp. 17–28, Jan. 2010, doi: 10.1002/stem.261.
45. W. K. Chau, C. K. Ip, A. S. C. Mak, H.-C. Lai, and A. S. T. Wong, "c-Kit mediates chemoresistance and tumor-initiating capacity of ovarian cancer cells through activation of Wnt/β-catenin-ATP-binding cassette G2 signaling," Oncogene, vol. 32, no. 22, pp. 2767–2781, May 2013, doi: 10.1038/onc.2012.290.
46. L. Luo et al., "Ovarian cancer cells with the CD117 phenotype are highly tumorigenic and are related to chemotherapy outcome," Exp. Mol. Pathol., vol. 91, no. 2, pp. 596–602, Oct. 2011, doi: 10.1016/j.yexmp.2011.06.005.
47. S. Zhang et al., "Identification and characterization of ovarian cancer-initiating cells from primary human tumors," Cancer Res., vol. 68, no. 11, pp. 4311–4320, Jun. 2008, doi: 10.1158/0008-5472.CAN-08-0364.
48. T. Nunes et al., "Targeting Cancer Stem Cells to Overcome Chemoresistance," Int. J. Mol. Sci., vol. 19, no. 12, Art. no. 12, Dec. 2018, doi: 10.3390/ijms19124036.
49. A. Malanchi et al., "Interactions between cancer stem cells and their niche govern metastatic colonization," Nature, vol. 481, no. 7379, pp. 85–89, Jan. 2012, doi: 10.1038/nature10694.
50. G.-B. Jang et al., "Blockade of Wnt/β-catenin signaling suppresses breast cancer metastasis by inhibiting CSC-like phenotype," Sci. Rep., vol. 5, no. 1, Art. no. 1, Jul. 2015, doi: 10.1038/srep12465.
51. S. Patskovsky, E. Bergeron, and M. Meunier, "Hyperspectral darkfield microscopy of PEGylated gold nanoparticles targeting CD44-expressing cancer cells," J. Biophotonics, vol. 8, no. 1–2, pp. 162–167, 2015, doi: 10.1002/jbio.201300165.
52. S. Liang et al., "CD44v6 Monoclonal Antibody-Conjugated Gold Nanostars for Targeted Photoacoustic Imaging and Plasmonic Photothermal Therapy of Gastric Cancer Stem-like Cells," Theranostics, vol. 5, no. 9, pp. 970–984, 2015, doi: 10.7150/thno.11632.
53. A. R. Burke et al., "The resistance of breast cancer stem cells to conventional hyperthermia and their sensitivity to nanoparticle-mediated photothermal therapy," Biomaterials, vol. 33, no. 10, pp. 2961–2970, Apr. 2012, doi: 10.1016/j.biomaterials.2011.12.052.
54. A. S. Cazet et al., "Targeting stromal remodeling and cancer stem cell plasticity overcomes chemoresistance in triple negative breast cancer," Nat. Commun., vol. 9, no. 1, p. 2897, Jul. 2018, doi: 10.1038/s41467-018-05220-6.
55. M. J. Bissell and D. Radisky, "Putting tumours in context," Nat. Rev. Cancer, vol. 1, no. 1, pp. 46–54, Oct. 2001, doi: 10.1038/35094059.
56. D. Hong et al., "AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer," Sci. Transl. Med., vol. 7, no. 314, p. 314ra185, Nov. 2015, doi: 10.1126/scitranslmed.aac5272.
57. A. Tsuyada et al., "CCL2 mediates cross-talk between cancer cells and stromal fibroblasts that regulates breast cancer stem cells," Cancer Res., vol. 72, no. 11, pp. 2768–2779, Jun. 2012, doi: 10.1158/0008-5472.CAN-11-3567.
58. X. Ding et al., "Effects of NOTCH1 signaling inhibitor γ-secretase inhibitor II on growth of cancer stem cells," Oncol. Lett., vol. 16, no. 5, pp. 6095–6099, Nov. 2018, doi: 10.3892/ol.2018.9377.
59. S. Zhang et al., "CD133(+)CXCR4(+) colon cancer cells exhibit metastatic potential and predict poor prognosis of patients," BMC Med., vol. 10, p. 85, Aug. 2012, doi: 10.1186/1741-7015-10-85.
60. M. Huang, Y. Li, H. Zhang, and F. Nan, "Breast cancer stromal fibroblasts promote the generation of CD44+CD24- cells through SDF-1/CXCR4 interaction," J. Exp. Clin. Cancer Res. CR, vol. 29, no. 1, p. 80, Jun. 2010, doi: 10.1186/1756-9966-29-80.
61. W. Xiao, Z. Gao, Y. Duan, W. Yuan, and Y. Ke, "Notch signaling plays a crucial role in cancer stem-like cells maintaining stemness and mediating chemotaxis in renal cell carcinoma," J. Exp. Clin. Cancer Res. CR, vol. 36, no. 1, p. 41, Mar. 2017, doi: 10.1186/s13046-017-0507-3.

62. D. E. Johnson, R. A. O'Keefe, and J. R. Grandis, "Targeting the IL-6/JAK/STAT3 signalling axis in cancer," Nat. Rev. Clin. Oncol., vol. 15, no. 4, pp. 234–248, Apr. 2018, doi: 10.1038/nrclinonc.2018.8.
63. K. J. Pienta et al., "Phase 2 study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 (CCL2), in metastatic castration-resistant prostate cancer," Invest. New Drugs, vol. 31, no. 3, pp. 760–768, Jun. 2013, doi: 10.1007/s10637-012-9869-8.
64. C. Even et al., "Safety and clinical activity of the Notch inhibitor, crenigacestat (LY3039478), in an open-label phase I trial expansion cohort of advanced or metastatic adenoid cystic carcinoma," Invest. New Drugs, vol. 38, no. 2, pp. 402–409, Apr. 2020, doi: 10.1007/s10637-019-00739-x.
65. M. W. den Hollander et al., "TGF-β Antibody Uptake in Recurrent High-Grade Glioma Imaged with 89Zr-Fresolimumab PET," J. Nucl. Med. Off. Publ. Soc. Nucl. Med., vol. 56, no. 9, pp. 1310–1314, Sep. 2015, doi: 10.2967/jnumed.115.154401.
66. M. Todaro et al., "CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis," Cell Stem Cell, vol. 14, no. 3, pp. 342–356, Mar. 2014, doi: 10.1016/j.stem.2014.01.009.
67. Y. Cheng et al., "Osteopontin Promotes Colorectal Cancer Cell Invasion and the Stem Cell-Like Properties through the PI3K-AKT-GSK/3β-β/Catenin Pathway," Med. Sci. Monit. Int. Med. J. Exp. Clin. Res., vol. 25, pp. 3014–3025, Apr. 2019, doi: 10.12659/MSM.913185.
68. L. Wang et al., "Oncolytic Herpes Simplex Virus and PI3K Inhibitor BKM120 Synergize to Promote Killing of Prostate Cancer Stem-like Cells," Mol. Ther. Oncolytics, vol. 13, pp. 58–66, Jun. 2019, doi: 10.1016/j.omto.2019.03.008.
69. Y. Liu, X. B. Zhang, J. J. Liu, S. Zhang, and J. Zhang, "[NVP-BKM120 in combination with letrozole inhibit human breast cancer stem cells via PI3K/mTOR pathway]," Zhonghua Yi Xue Za Zhi, vol. 99, no. 14, pp. 1075–1080, Apr. 2019, doi: 10.3760/cma.j.issn.0376-2491.2019.14.008.
70. P. Schöffski et al., "A phase Ib study of pictilisib (GDC-0941) in combination with paclitaxel, with and without bevacizumab or trastuzumab, and with letrozole in advanced breast cancer," Breast Cancer Res. BCR, vol. 20, no. 1, p. 109, Sep. 2018, doi: 10.1186/s13058-018-1015-x.
71. A. Wicki et al., "First-in human, phase 1, dose-escalation pharmacokinetic and pharmacodynamic study of the oral dual PI3K and mTORC1/2 inhibitor PQR309 in patients with advanced solid tumors (SAKK 67/13)," Eur. J. Cancer Oxf. Engl. 1990, vol. 96, pp. 6–16, Jun. 2018, doi: 10.1016/j.ejca.2018.03.012.
72. Y. Ando et al., "Phase I study of alpelisib (BYL719), an α-specific PI3K inhibitor, in Japanese patients with advanced solid tumors," Cancer Sci., vol. 110, no. 3, pp. 1021–1031, Mar. 2019, doi: 10.1111/cas.13923.
73. S. J. Hotte et al., "A Phase II Study of PX-866 in Patients with Recurrent or Metastatic Castration-resistant Prostate Cancer: Canadian Cancer Trials Group Study IND205," Clin. Genitourin. Cancer, vol. 17, no. 3, pp. 201-208.e1, Jun. 2019, doi: 10.1016/j.clgc.2019.03.005.
74. "Pharmacologic Wnt Inhibition Reduces Proliferation, Survival, and Clonogenicity of Glioblastoma Cells - PubMed." https://pubmed.ncbi.nlm.nih.gov/26222502/ (accessed Dec. 29, 2022).
75. Y. Cheng et al., "Wnt-C59 arrests stemness and suppresses growth of nasopharyngeal carcinoma in mice by inhibiting the Wnt pathway in the tumor microenvironment," Oncotarget, vol. 6, no. 16, pp. 14428–14439, Jun. 2015, doi: 10.18632/oncotarget.3982.
76. G. Wang et al., "Cyclophilin A Maintains Glioma-Initiating Cell Stemness by Regulating Wnt/β-Catenin Signaling," Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res., vol. 23, no. 21, pp. 6640–6649, Nov. 2017, doi: 10.1158/1078-0432.CCR-17-0774.
77. C. Wu, S. Hu, J. Cheng, G. Wang, and K. Tao, "Smoothened antagonist GDC-0449 (Vismodegib) inhibits proliferation and triggers apoptosis in colon cancer cell lines," Exp. Ther. Med., vol. 13, no. 5, pp. 2529–2536, May 2017, doi: 10.3892/etm.2017.4282.
78. W. Tong et al., "GANT-61 and GDC-0449 induce apoptosis of prostate cancer stem cells through a GLI-dependent mechanism," J. Cell. Biochem., vol. 119, no. 4, pp. 3641–3652, Apr. 2018, doi: 10.1002/jcb.26572.
79. G. Valenti et al., "Cancer Stem Cells Regulate Cancer-Associated Fibroblasts via Activation of Hedgehog Signaling in Mammary Gland Tumors," Cancer Res., vol. 77, no. 8, pp. 2134–2147, Apr. 2017, doi: 10.1158/0008-5472.CAN-15-3490.
80. P. Jagust, B. de Luxán-Delgado, B. Parejo-Alonso, and P. Sancho, "Metabolism-Based Therapeutic Strategies Targeting Cancer Stem Cells," Front. Pharmacol., vol. 10, 2019, Accessed: Oct. 27, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fphar.2019.00203
81. J. Wei, J. Sun, and Y. Liu, "Enhanced targeting of prostate cancer-initiating cells by salinomycin-encapsulated lipid-PLGA nanoparticles linked with CD44 antibodies," Oncol. Lett., vol. 17, no. 4, pp. 4024–4033, Apr. 2019, doi: 10.3892/ol.2019.10050.
82. L. Yang et al., "Targeting cancer stem cell pathways for cancer therapy," Signal Transduct. Target. Ther.,

vol. 5, no. 1, pp. 1–35, Feb. 2020, doi: 10.1038/s41392-020-0110-5.
83. H. Lv et al., "Vitamin C preferentially kills cancer stem cells in hepatocellular carcinoma via SVCT-2," Npj Precis. Oncol., vol. 2, no. 1, Art. no. 1, Jan. 2018, doi: 10.1038/s41698-017-0044-8.
84. J. R. Brown et al., "Phase II clinical trial of metformin as a cancer stem cell-targeting agent in ovarian cancer," JCI Insight, vol. 5, no. 11, p. 133247, Jun. 2020, doi: 10.1172/jci.insight.133247.
85. E. Quaglino, L. Conti, and F. Cavallo, "Breast cancer stem cell antigens as targets for immunotherapy," Semin. Immunol., vol. 47, p. 101386, Feb. 2020, doi: 10.1016/j.smim.2020.101386.
86. Y. Gu, X. Zhao, and X. Song, "Ex vivo pulsed dendritic cell vaccination against cancer," Acta Pharmacol. Sin., vol. 41, no. 7, Art. no. 7, Jul. 2020, doi: 10.1038/s41401-020-0415-5.
87. R. Su et al., "Targeting FTO Suppresses Cancer Stem Cell Maintenance and Immune Evasion," Cancer Cell, vol. 38, no. 1, pp. 79-96.e11, Jul. 2020, doi: 10.1016/j.ccell.2020.04.017.
88. "PD-1 blockade enhances the antitumor efficacy of GM-CSF surface-modified bladder cancer stem cells vaccine - Shi - 2018 - International Journal of Cancer - Wiley Online Library." https://onlinelibrary.wiley.com/doi/full/10.1002/ijc.31219 (accessed Nov. 04, 2022).
89. T.-T. Liao, C.-C. Lin, J.-K. Jiang, S.-H. Yang, H.-W. Teng, and M.-H. Yang, "Harnessing stemness and PD-L1 expression by AT-rich interaction domain-containing protein 3B in colorectal cancer," Theranostics, vol. 10, no. 14, pp. 6095–6112, May 2020, doi: 10.7150/thno.44147.
90. A. Emami Nejad et al., "The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment," Cancer Cell Int., vol. 21, no. 1, p. 62, Jan. 2021, doi: 10.1186/s12935-020-01719-5.