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REVIEW ARTICLE

Drug repurposing: A novel strategy to target cancer stem cells and therapeutic resistance

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Abstract Chemotherapy is an effortless and frequently used approach in cancer therapy. However, in most cases, it can only prolong life expectancy and does not guarantee a complete cure. Furthermore, chemotherapy is associated with severe adverse effects, one of the major complications of effective cancer therapy. In addition, newly published research outputs show that cancer stem cells are involved in cancer disease progression, drug resistance, metastasis, and recurrence and that they are functional in the trans-differentiation capacity of cancer stem cells to cancer cells in response to treatments. Novel strategies are therefore required for better management of cancer therapy. The prime approach would be to synthesize and develop novel drugs that need extensive resources, time, and endurance to be brought into therapeutic use. The subsequent approach would be to screen the anti-cancer activity of available non-cancerous drugs. This concept of repurposing non-cancer drugs as an alternative to current cancer therapy has become popular in recent years because using existing anticancer drugs has several adverse effects. Micronutrients have also been investigated for cancer therapy due to their significant anti-cancer effects with negligible or no side effects and availability in food sources. In this paper, we discuss an ideal hypothesis for screening available non-cancerous drugs with anticancer activity, with a focus on cancer stem cells and their clinical application for cancer treatment. Further, drug repurposing and the combination of micronutrients that can target both cancers and cancer stem cells may result in a better therapeutic approach leading to maximum tumor growth control.

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Abbreviations

ATRA	all-transretinoic acid
CEA	carcinoma embryonic antigen
CSCs	cancer stem cells
DNA	deoxyribonucleic acid
EGCG	epigallocatechin gallate
EGFR	epidermal growth factor receptor
EMA	epithelial membrane antigen
EMT	epithelial to mesenchymal transition
EpCAM	epithelial cell adhesion molecule
FDA	Food and Drug Administration
FRB	FKBP-rapamycin binding
MAbs	monoclonal antibodies
MDR1	multi-drug resistant protein-1
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
ROR	receptor tyrosine kinase-like orphan receptor
STAT	signal transducer and activator of transcription
TAAs	tumor-associated antigens
TGFβ	transforming growth factor β
UK	United Kingdom
USA	United States of America
VEGF	vascular endothelial growth factor

Introduction

Cancer is still a leading cause of death worldwide and accounted for nearly 10 million deaths in 2020 as per the world cancer reports 2020.[1] The high incidence rate of cancer may be due to several reasons, such as a mutation in genes, environmental factors, inadequate physical activity, different lifestyles, unstable eating-related behaviors, smoking, and alcohol consumption.[2–6] Present therapy options for treating different cancers at various stages of the disease are chemotherapy, radiation, and surgery for solid tumors or a combination of these.[7] Although these are effective in reducing the indicated cancer, they are associated with drawbacks and numerous side effects. Radiation therapy involves changing the microenvironment of the tumor cells where the surrounding healthy cells may also undergo DNA damage leading to a new cancer incidence.[8] Similarly, surgical therapy is also a primary treatment modality for solid tumors; it strongly affects the survival of patients. However, its success rate depends on the surgeon's expertise and screening methods that involve imaging facilities at the hospital.[9] Chemotherapy uses drugs intended to cure cancer, lessening the chance of its return and easing cancer symptoms that are causing pain and other harmful effects.[10] Chemo works by either stopping or slowing the growth of fast-growing cancer cells but can exert toxic effects on normal cells as well.[11] Injury and toxicity to normal cells surrounding the malignant cells are the possible risks associated with current treatment strategies, including chemotherapy, surgery, and radiation therapy.[12–14] Therefore, it is important to focus on newer advanced treatment modalities through which cancer cells are killed with fewer or no side effects. Research and clinical trials on some novel modalities, for example, sonodynamic, photodynamic therapies, immunotherapy, and virotherapy, are explored to improve current treatment outcomes. However, these new strategies have been tried against cancer cells but have not greatly focused on specifically targeting cancer stem cells (CSCs).[15–19]

Even though constant efforts are in progress to minimize disease progression and prolong life expectancy, the complete cure is still questionable. The prime reason for this might be that cancer is characterized by the eventual development of resistance or lack of response to these drugs and medications when treated with chemotherapy.[20] Recent literature has revealed that CSCs are present in any tumor with the capacity to self-renew and give different lineages of cancer cells that may be involved in the formation of the tumor. Further, CSCs in the tumor can cause proliferation, aggressiveness, and resistance to treatment.[21] Thus, targeting the CSCs and therapeutic resistance is a novel approach for restricting the disease progression and improving the therapy. As most of the existing chemotherapeutics are associated with several serious side effects and can fight the bulk tumor populations and not the CSCs, the design of new drugs against cancer as well as CSCs is an urgent requirement.[22] However, developing new drugs has to overcome several hurdles to bring the drug into the market. It is very expensive and time-consuming to carry out all types of clinical trials.[23]

This review emphasizes old drug candidates, which are the drugs approved, found safe, and already in use for treating non-cancerous diseases with anti-cancer activity. These drugs can also be explored to target the CSCs for therapeutic application. This approach may bring novel anti-cancer agents as they are already approved and found safe with known clinical data such as efficacy and safety. Further, we discuss those drugs used in combination with other known anti-cancer agents, preferably phytonutrients or micronutrients, for better therapy with fewer or negligible side effects.

Need for targeting CSCs

The high recurrence rate of cancer after the tumor treatment remains a significant challenge for successfully eradicating tumor prognosis in different cancer types.[24] The reason behind this may be the CSCs and chemoresistance.[25] CSCs are well-defined as a small subpopulation of cancer cells within tumors with self-renewal, differentiation, and metastasis/malignant potential. Their biological characteristics are involved in tumor initiation and development, metastasis, and recurrence.[26] Therapeutics that are aimed to target the tumor population alone has no or negligible effect on CSCs. CSCs, due to their stemness and therapeutic resistance, cause tumor relapse. So, it is necessary to destroy the CSC population responsible for differentiation and proliferation, by targeting CSCs that may lead to noticeable persistent tumoral regression.[27]

Origin of CSCs

CSCs are believed to be developed from differentiated adult cells, tissue-resident stem cells, or their progeny

upon transformation and self-renewal.[28] In general, transformation can occur during tissue regeneration and is additionally initiated and/or accelerated as a response to various infections, toxins, and therapies such as radiation.[29] Overexpression of oncogenes and/or inactivation of tumor suppressors lead to uncontrolled cell growth; hence, the differentiated cell undergoes de-differentiation and acquires stem cell characteristics.[30,31] In the case of stem cells and their progeny, as they possess indefinite growth potential, minute genomic changes can lead to mutations and irregular cell divisions.[32] Conventional cancer treatment targets the bulk of the tumor and cannot target CSCs due to their high resistance nature, leading to metastasis and tumor recurrence/relapse. Due to the plasticity of CSCs, it is beneficial to target CSC pathways along with conventional chemotherapeutics for a better therapeutic effect that improves the clinical outcomes of cancer therapy due to maximum possible tumor regression[33] (Fig. 1).

The identification of specific CSC surface markers, and the isolation and characterization of CSCs from bulk tumor population, helps in the development of strategies for the targeted eradication of CSCs. Research findings suggest that treating cancers with special emphasis on targeting CSCs appear to be an interesting area in cancer research.[34] Several stem cell markers, such as CD24, CD34, CD44, CD123, CD133, Oct4, Sox2, Nanog, c-kit, ABCG2, and aldehyde dehydrogenase (ALDH), have been identified in the CSC populations isolated from different tumor types and their expression is tissue type-specific as well as tumor subtype-specific.[35–37] Here we summarized different protein markers (antibodies) and their specific target cancer types in Table 1.

Features and biological roles of CSCs

It is believed that the biological characteristics of CSCs are accountable for tumorigenesis and cancer recurrence. CSCs have strong self-renewal ability, the ability to differentiate into different cell types, tumor invasion, and metastasis.

Self-renewal and differentiation

Self-renewal can be defined as the ability to regenerate, and it is the basic characteristic of CSCs by which they can generate more stem cells and differentiated cells. CSCs, through asymmetrical cell divisions, produce progenitor cells that retain stemness and cells that undergo subsequent post-mitotic differentiation.[53] The self-renewal ability of CSCs is the direct cause of tumorigenesis; hence, regulation of CSC self-renewal will provide a clear target for cancer treatment. CSCs can differentiate into different cell types and also transdifferentiate into other multilineage cells to regulate tumorigenesis.[54] CSCs share some of the same regulatory signaling pathways involving the self-renewal and differentiation process that the normal stem cells do, for example, the Wingless-related integration site (Wnt)/β-catenin, Sonic Hedgehog (Hdhg), and Notch pathways.[55]

Tumor invasion and metastasis

Metastasis is the process by which cancer cells travel from their primary site to other parts of the body through the blood or lymph system and form new tumors. The alteration of oncogenes, tumor suppressor genes, as well as those involved in DNA repair mechanisms, cause accumulated mutations leading to uncontrolled proliferation or tumorigenesis.[56] With no therapeutic intervention, cancer becomes increasingly progressive, and to compensate for the increasing need to survive, it begins to spread via a metastatic cascade. However, CSCs, even after therapeutic intervention, can increase aggression due to their resistance properties and subsequently re-initiate the tumor, causing tumor relapse. CSCs are closely related to epithelial-mesenchymal transformation (EMT), and stimulation of EMT promotes the invasion of tumor cells.[57] CSCs via interactions with the cellular components of the tumor microenvironment play a role in arranging the metastasis cascade by creating a growth-supportive niche and promoting angiogenesis. Thus, CSCs are the key "seeds" for tumor initiation and development, metastasis, and recurrence.[58]

Different signaling pathways involved in CSCs regulation

Various cell signaling pathways play a critical role in cancer therapy as the novel treatment strategies target these signaling pathways that are contributing to the cancer cells as well as the self-renewal processes of cancer stemness and differentiation capacity of CSCs for the growth of differentiated tumor cells. Some of the important pathways are briefly discussed.

Wnt/β-catenin pathway

Wnt pathway is involved in multiple biological processes, where they are involved in embryonic development, the genesis of an embryo, cell proliferation, and survival and development, along with regulating and expanding of CSCs.[59]

The Wnt pathway is a complex signaling pathway that includes 19 Wnt ligands, more than 12 receptors, and co-receptors that affect various cellular processes like polarity and cell fate.[60] The Wnt signaling pathway is of two types, canonical and noncanonical Wnt signaling. In canonical Wnt signaling, in the absence of Wnt ligands, β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β) through the FZD-LRP5/6 receptor complex, which leads to β-catenin degradation inhibiting β-catenin translocation from the cytoplasm to the nucleus.[61,62] In another way, in the presence of Wnt ligands, due to the binding of Wnt ligands with Frizzled (FZD) receptors and lung resistance-related protein (LRP) co-receptors, LRP receptors are phosphorylated by GSK3β and CK1α, and the β-Catenin is released from the Axin complex to enter the nucleus. Further, β-catenin activates the gene transcription through binding with LEF/TCF and enhancing the presence of histone-modifying coactivators, like BCL9, Pygo, CBP/p300,

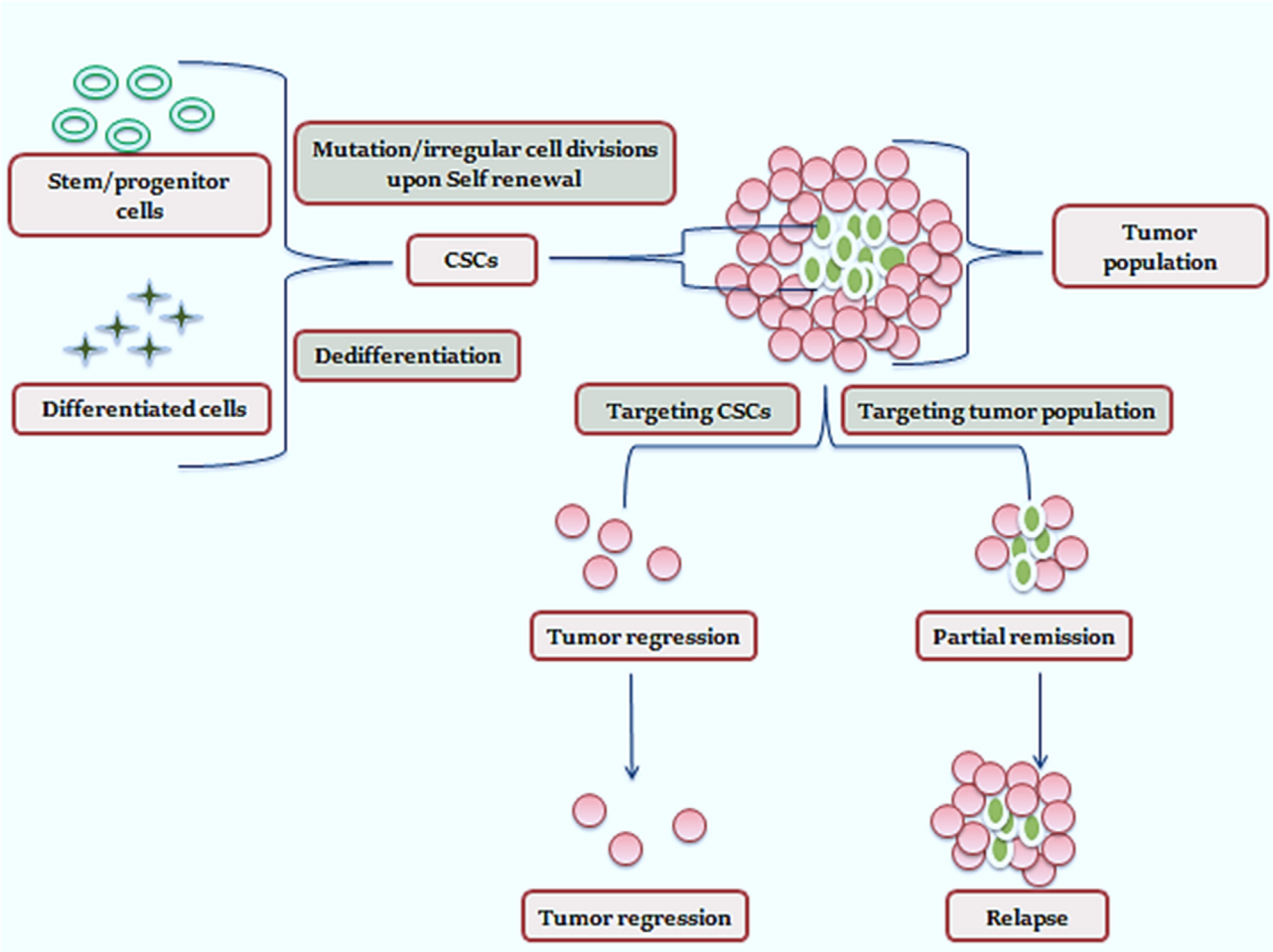

Figure 1 CSC (cancer stem cell) origin and treatment approach for cancer therapy.

Table 1 CSC markers (antibodies) were used to identify CSCs in different cancers.

No.	Surface marker	Targeting CSCs/cancer type
1	CD44, CD24, CD133, ESA	Pancreatic[38]
2	CD133, Bcrpa1, CD15	Brain[39]
3	CD44, CD24, CD133, CD13, CD90, EpCAM	Liver[40]
4	CD44, CK17, Brcp1	Cervical[41]
5	CD44, CD133, CD24, ABGC2, integrin $\alpha 2\beta 1$	Prostate[42]
6	CD133, CD47, CD44v6, EMA	Bladder[43]
7	CD96, Cd123, CD34	Leukaemia[44]
8	CD133, ABCG2, CD17	Lung[45]
9	CD133, ABCB5, CD20, CD271	Melanoma[46]
10	CD133, ABCB1, Brcp1	Ovarian[47]
11	CD133, CD44, BCRP1, ESA, CXCR1	Breast[48]
12	CD133, CD166, CD29, ESA, CD26, CD24	Colon[49]
13	CD133, CD44, CD90, ABCG2, ABCB1	Gastric[50,51]
14	CD133, CD44	Head and neck[52]

and BRG1.[63,64] Whereas noncanonical Wnt signaling does not involve β-catenin but the FZD receptors and/or ROR1/ROR2/RYK co-receptors activate PCP, RTK, or Ca^{2+} signaling cascades.[65] The difference between the Wnt pathway in the presence and absence of Wnt ligands was elucidated in Figure 2.

Notch signaling pathway

The receptor Notch is essential for fundamental cellular functions, including proliferation, differentiation, and apoptosis, and is implicated in malignant transformation.[66] It operates both in normal as well as CSCs populations where, upon activation by transmembrane proteins known as Delta-like and Jagged ligands, the Notch receptor will be translocated to the nucleus and associated with a DNA-bound protein, starting events of a cascade of transduction in the cell (Fig. 3). Notch activation contributes to the development of a variety of stem and early progenitor cells.[67] The defective Notch signaling is implicated in many cancers and diseases, including T-cell acute lymphoblastic leukemia, multiple sclerosis, melanoma, breast cancer, meningioma, lung adenocarcinoma, and many other diseases due to abnormal proliferation, reduced differentiation, and arrested apoptosis.[68]

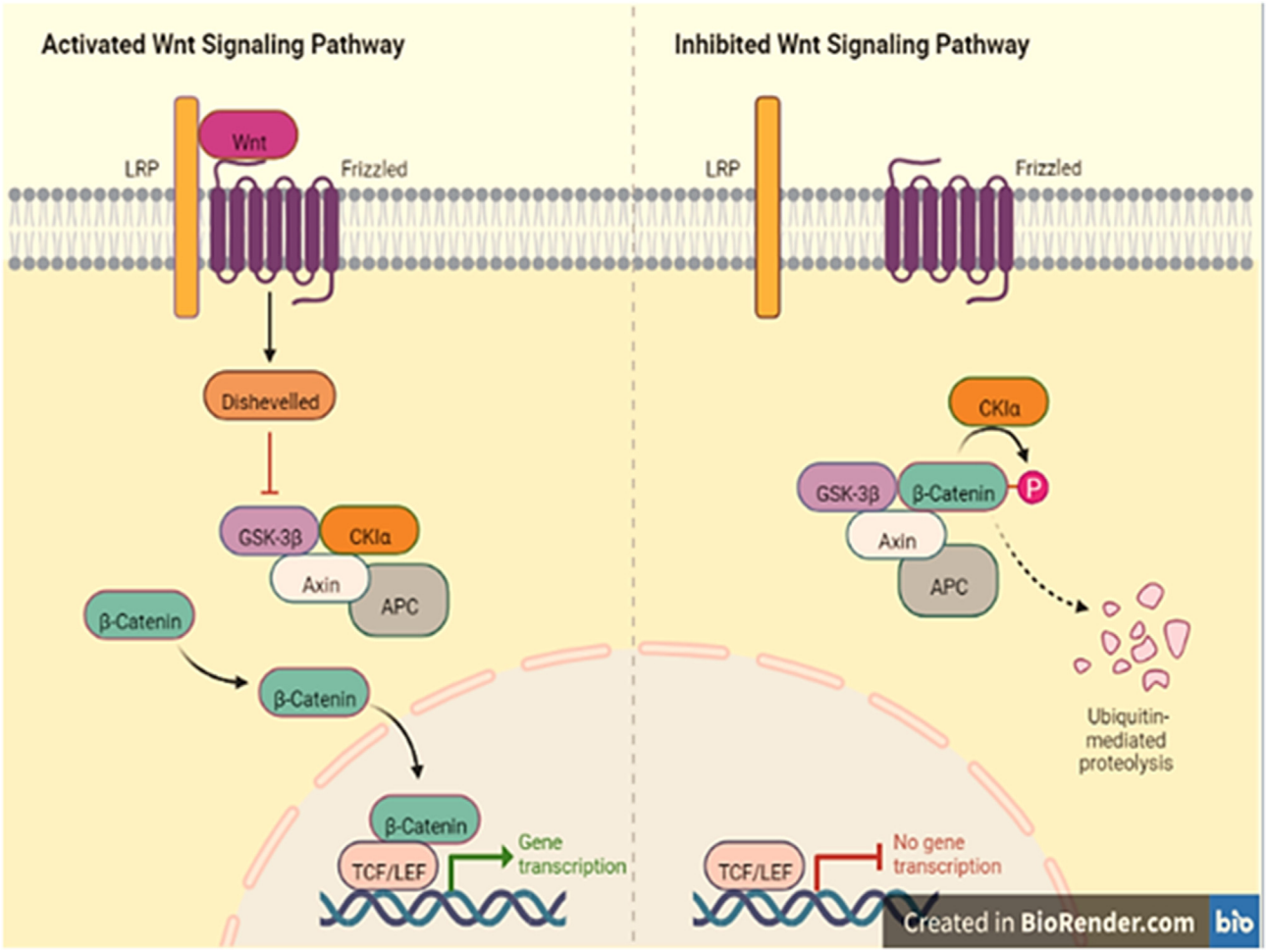

Figure 2 Difference between Wnt pathway in the presence and absence of Wnt ligands (the image was recreated using BioRender.com).

The Hdhg signaling pathway

The Hdhg pathway contributes a major part to stem-cell maintenance in the process of embryonic development and patterning.[69] It is also involved in regulating target genes that take part in various cellular functions like cell survival, cell proliferation, metastasizing, and autoregulation of different pathways.[70] The Hdhg signaling pathway is elucidated in Figure 4.

Smad/transforming growth factor-beta (TGF-β) signaling pathway

TGF-β signaling pathway regulates a wide range of cellular processes, including cell growth, differentiation, migration, apoptosis, homeostasis, *etc.*, during embryonic development as well as in the adult organism.[71] TGFβ superfamily ligands bind to a type II (TβRII) receptor, which recruits and phosphorylates a type I (TβRI) receptor. The TβRI then phosphorylates receptor-regulated Smad proteins (R-SMADs), Smad2/3 for TGF-β and activin signaling, while Smad1/5/8 for BMP signaling, which can now bind to form a heterocomplex with the coSMAD SMAD4. These R-SMAD/coSMAD complexes accumulate in the nucleus and act as transcription factors for regulating target gene expression (Fig. 5)[72,73]. TGF-β pathway was studied for its role in stem cells' self-renewal and carcinogenesis in a blood-regulated organ and the interleukin 6 (IL-6) signaling pathway involved in activating STAT-3 in hepatic stem cells.[74] These pathways can be considered while screening existing drugs available for their newer anti-cancer activity, which may help to bring the potential anti-cancer activity of screening new compounds.[75]

Mechanisms of chemoresistance

The most involved molecular mechanisms of chemoresistance are discussed hereunder and illustrated in Figure 6.

Drug inactivation

Several anti-cancer drugs are pro-carcinogenic and must undergo metabolic activation mostly through the cytochrome P450 (CYP) system, glutathione-S-transferase (GST) superfamily, and uridine diphospho-glucuronosyltransferase (UGT) superfamily to obtain clinical efficacy.[76] However, mutations in cancer cells and CSCs can alter their metabolic

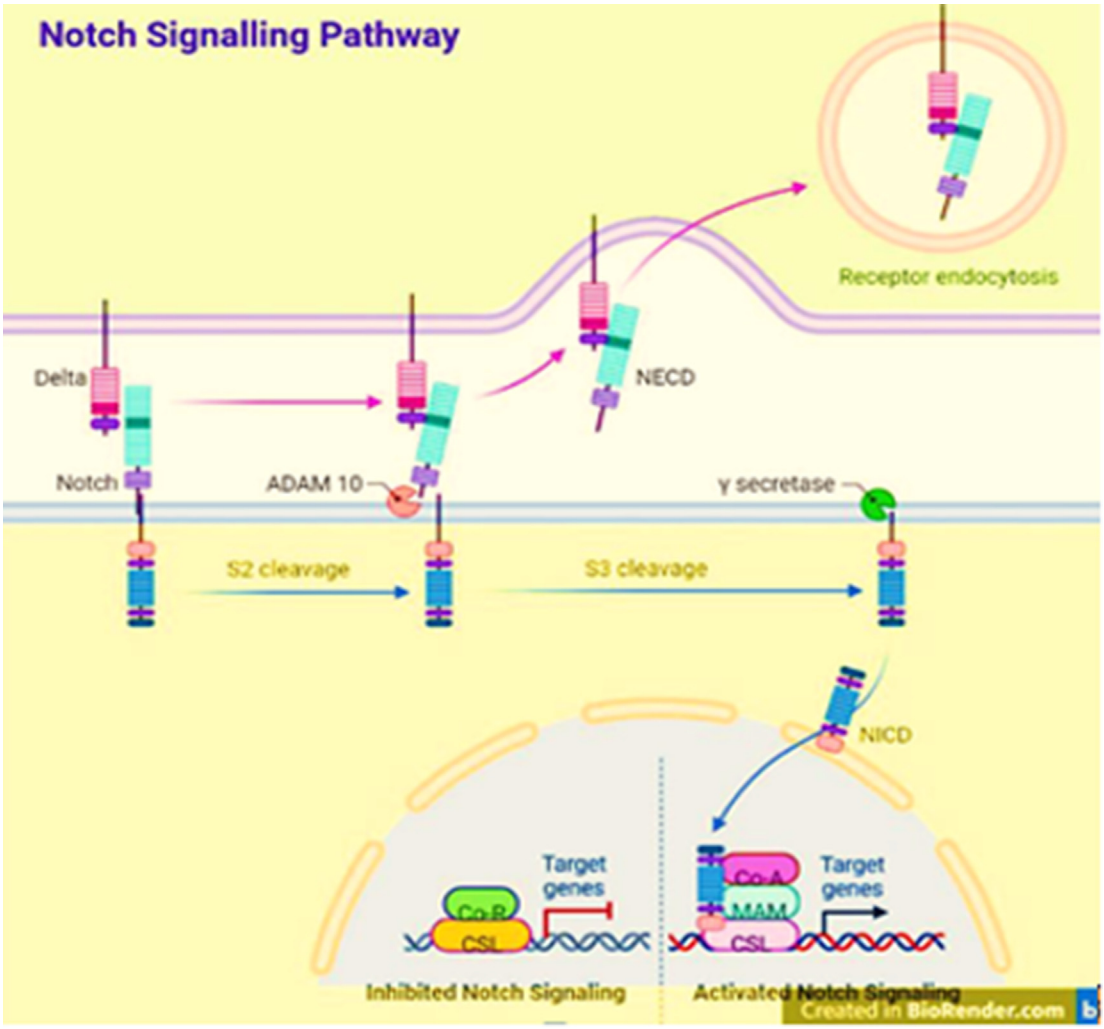

Figure 3 Notch signaling pathway (the image was recreated using BioRender.com).

capabilities and suppress drug activation leading to resistance to chemotherapy.[77] Another mechanism of developing resistance via drug inactivation is changing in the apoptosis-related proteins such as tumor suppressor protein p53 (TP53). TP53 is mutated in 50% of cancers, making it non-functional, followed by chemoresistance.[78,79] Furthermore, the inactivation of P53 regulators, for example, caspase-9 and its cofactor, apoptotic protease activating factor 1 (Apaf-1), also lead to chemoresistance.[80]

Alteration of drug target

Certain anti-cancer drugs target specific target sites to cause anti-cancer effects by diverse mechanisms such as DNA damage, inhibition of DNA synthesis, and/or halting mitotic processes.[81] However, alterations in specific drug targets due to mutations or changes in their expression levels at drug target sites can alter drug responses in cancer and CSCs and develop chemoresistance.[82] Drug target alterations like mutations in beta-tubulin resulted in taxane resistance in ovarian cancers.[83,84] Overexpression of Human epidermal growth factor receptor 2 (HER2) resulted in chemoresistance upon long-term usage of HER2 inhibitors in 30% of breast cancer patients.[85,86] Genomic amplification of androgen receptors enables resistance to leuprolide and bicalutamide used for androgen deprivation therapy in 30% of prostate cancers. Other genetic alterations, such as chromosomal rearrangements and mutations in anaplastic lymphoma kinase, are seen in anaplastic large-cell lymphoma.[86,87]

Efflux mechanism

The efflux mechanism is one of the most studied mechanisms of cancer drug resistance and involves reducing drug accumulation by enhancing efflux.[88] ATP- binding cassette (ABC) transporters ABCB1 (P-gp or MDR1), ABCG2 (BCRP), and ABCC1 (MRP1) are the most studied membrane proteins that transport various substances across cellular membranes and are implicated in various chemoresistant cancers such as high levels of MRP1 in neuroblastoma, increased expression of BCRP in small cell lung cancer and also expressed in stem cells.[89,90] In healthy cells, this efflux mechanism prevents overaccumulation of toxins, protects the body by pumping drugs and other harmful molecules out, and contributes to the maintenance of the blood—brain barrier, whereas, in

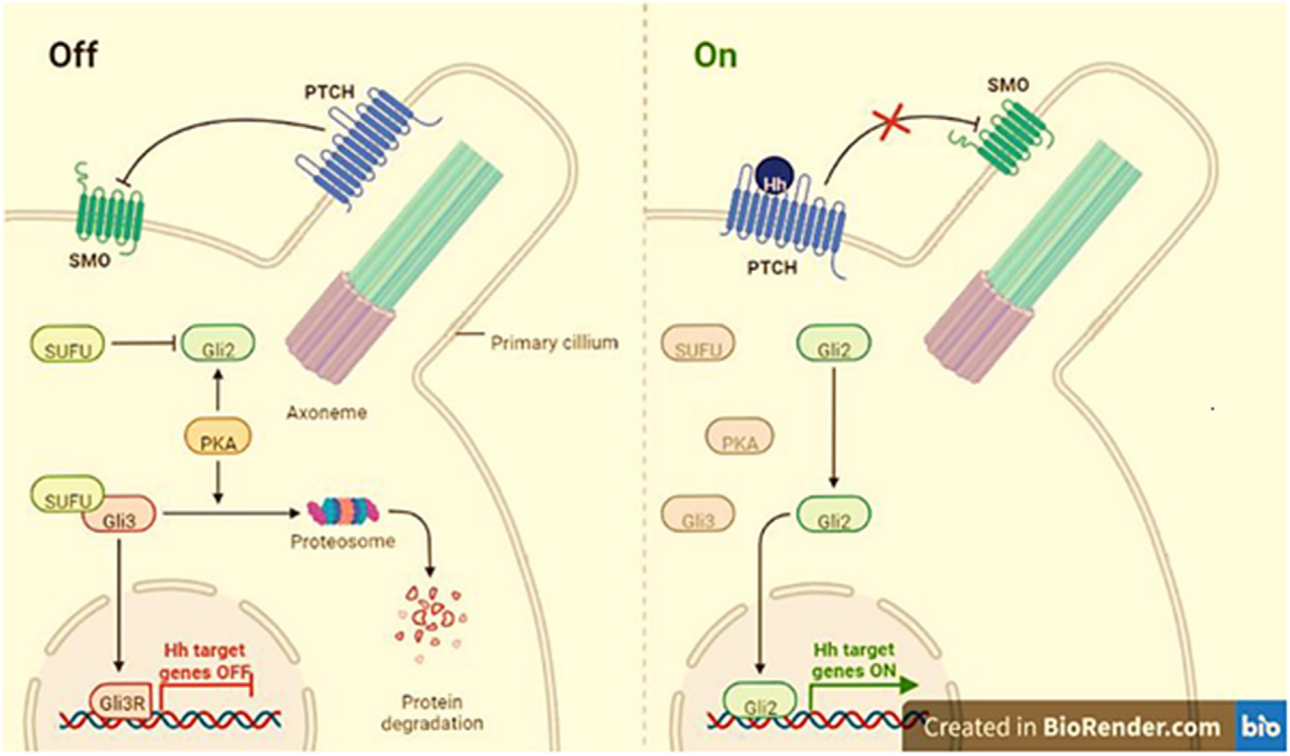

Figure 4 The Hdhg signaling pathway (the image was recreated using BioRender.com).

cancer cells, it acts as a mechanism for drug resistance by protecting cancer cells and CSCs from many first-line chemotherapies.[91]

The repair of damaged DNA

Any processes interfering with the DNA damage mechanisms can reverse the drug-induced damage as most chemotherapy drugs directly or indirectly act by damaging DNA and affect the efficacy of DNA-damaging cytotoxic drugs.[92] For example, cisplatin causes harmful DNA cross-links, leading to apoptosis. The DNA repair mechanisms, namely nucleotide excision repair and homologous recombination, are the primary DNA repair mechanisms that reverse cisplatin-caused damage.[93] Hence, it is suggested that a combination of repair pathway inhibitors with DNA-damaging chemotherapy can increase the efficacy of the chemotherapy.

Cell death inhibition

Apoptosis and autophagy are two important regulatory events contributing to cell death. Chemoresistance to those anti-cancer drugs that act by inducing cell death can be developed through the altered expression or regulation of cell death pathways.[94]

Apoptosis is responsible for the programmed cell death via activation, expression, and regulation of a variety of genes and maintains a stable internal environment. This involves various proteins such as B-cell lymphoma 2 (BCL-2) proteins, Akt, cysteinyl, and aspartate-specific proteases (caspases), among which 18, 2, 3, 6, 7, 8, 9, and 10 are known as apoptotic caspases and death receptor ligands on the cell surface.[95] Caspases 8 and 10 in the extrinsic pathway, whereas caspase 9 in the intrinsic pathway, are vital. These two pathways collectively activate the caspase-3 downstream, causing apoptosis.[96] Besides these two pathways, apoptosis can also occur through a caspase-independent pathway that depends on apoptosis-inducing factors, flavoproteins in the inner mitochondrial membrane causing pro-apoptosis. Modifications in the level of expression of BCL-2 proteins and other anti-apoptosis proteins and abnormal activity of downstream transcription modulators such as NF-κB and STAT result in cell death inhibition caused by various chemotherapeutics in different cancer types.[97,98]

Autophagy, in normal cells, is a self-digestion process that involves the degradation of proteins, organelles, and cells supplying nutrients and energy required for cell survival. However, the tumor microenvironment promotes tumor growth by providing nutrients to cancer cells. Further, the cross-talk between autophagy and apoptosis leads to cell death inhibition.[99]

Epigenetic mechanisms

Alterations in the epigenetic mechanisms, including DNA methylation and histone modifications, affect the regulation of CSC features and contribute to tumor progression, metastasis, and therapeutic resistance.

DNA methylation is a vital regulatory mechanism for gene expression when a methyl group is covalently added to the C-5 position of DNA cytosine rings by DNA methyltransferases which is altered in carcinogenesis.[100] DNA hypermethylation

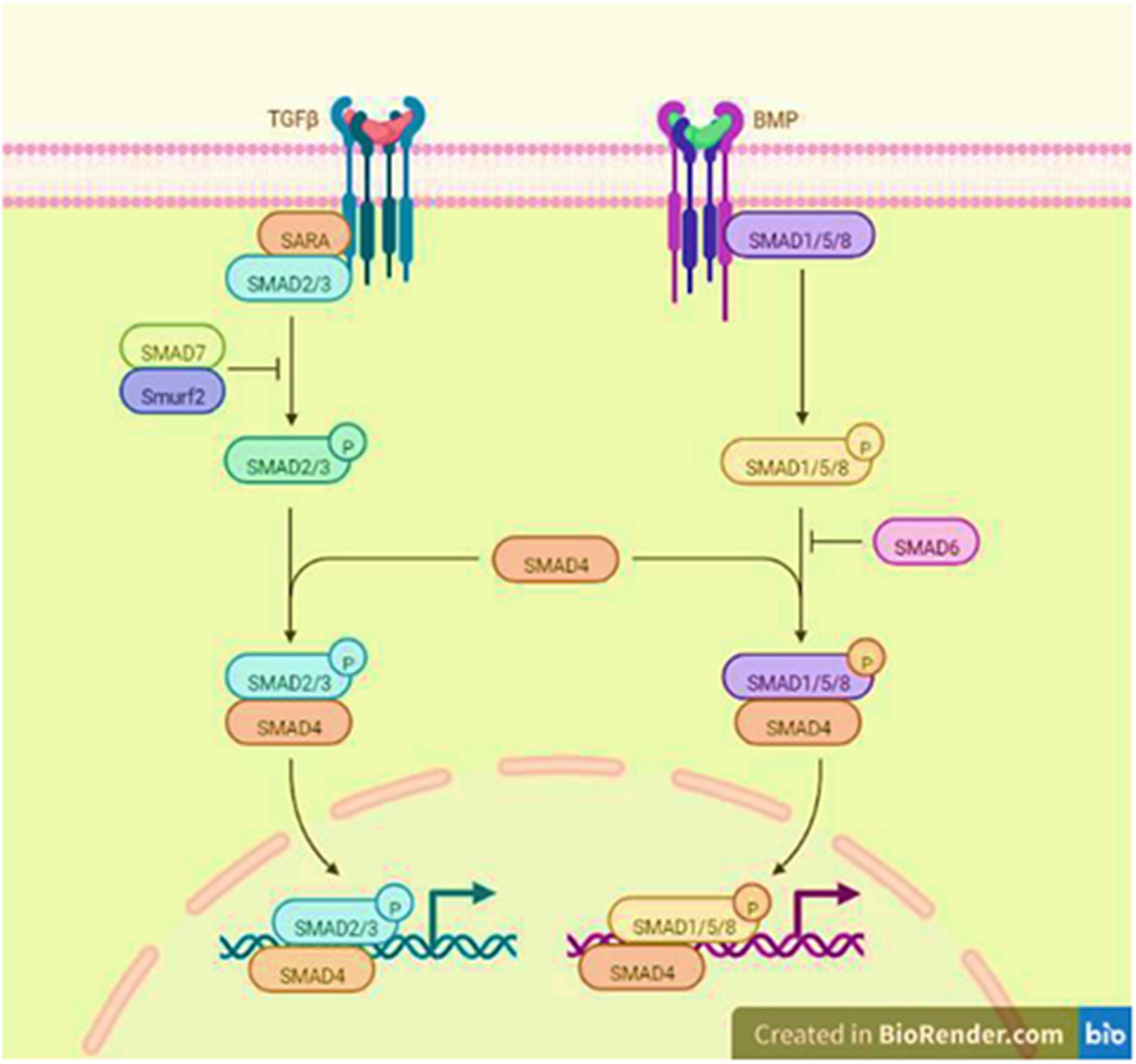

Figure 5 Smad/TGF-β signaling pathway (the image was recreated using BioRender.com).

at the promotor region controls MDR1 transcription and decreases gene expression. DNA hypermethylation, along with chromatin hypoacetylation can lead to the silencing of tumor-suppressor genes such as retinoblastoma 1 (RB1), BRCA1, CDKN2A (p16), *etc*. in colorectal carcinomas.[101,102] DNA hypomethylation contributes to chromosomal instability and tumor progression, which can often be observed at the early stages of tumor development. Furthermore, DNA hypomethylation can result in the activation of specific key genes involved in tumorigenesis; for example, the TERT gene encoding telomerase reverse transcriptase upon promoter methylation increases transcription and protein expression. Mutations in the TERT gene are associated with an increased risk of different cancer types, predominantly melanoma and acute myeloid leukemia.[103,104]

The nucleosomes, the basic chromatin units, consist of four core histone proteins, H3, H4, H2A, and H2B, forming an octamer that is enveloped with a 147-base-pair segment of DNA. The amino-terminal tails of histones are subjected to a variety of covalent post-translational modifications which facilitate several critical biological processes, generally via chromatin modification causing either expression or suppression of target genes that in turn, alter the chromatin structure and function.[105] Histone modifications happen by several means, including acetylation, methylation, phosphorylation, citrullination, ubiquitination, ADP-ribosylation, deamination, formylation, O-GlcNAcylation, propionylation, butyrylation, crotonylation, and proline isomerization. However, acetylation, methylation, and phosphorylation are widely studied.[106] In addition to DNA methylation and histone modifications, small non-coding RNAs or miRNAs with 19—25 nucleotides length were found to regulate mRNAs at the post-transcriptional level, and abnormal expression of some of them is related to tumor growth and metastasis.[107] They were also found to be regulated by promoter DNA methylation, thus making epigenetic regulation of tumorigenesis complicate.[108,109]

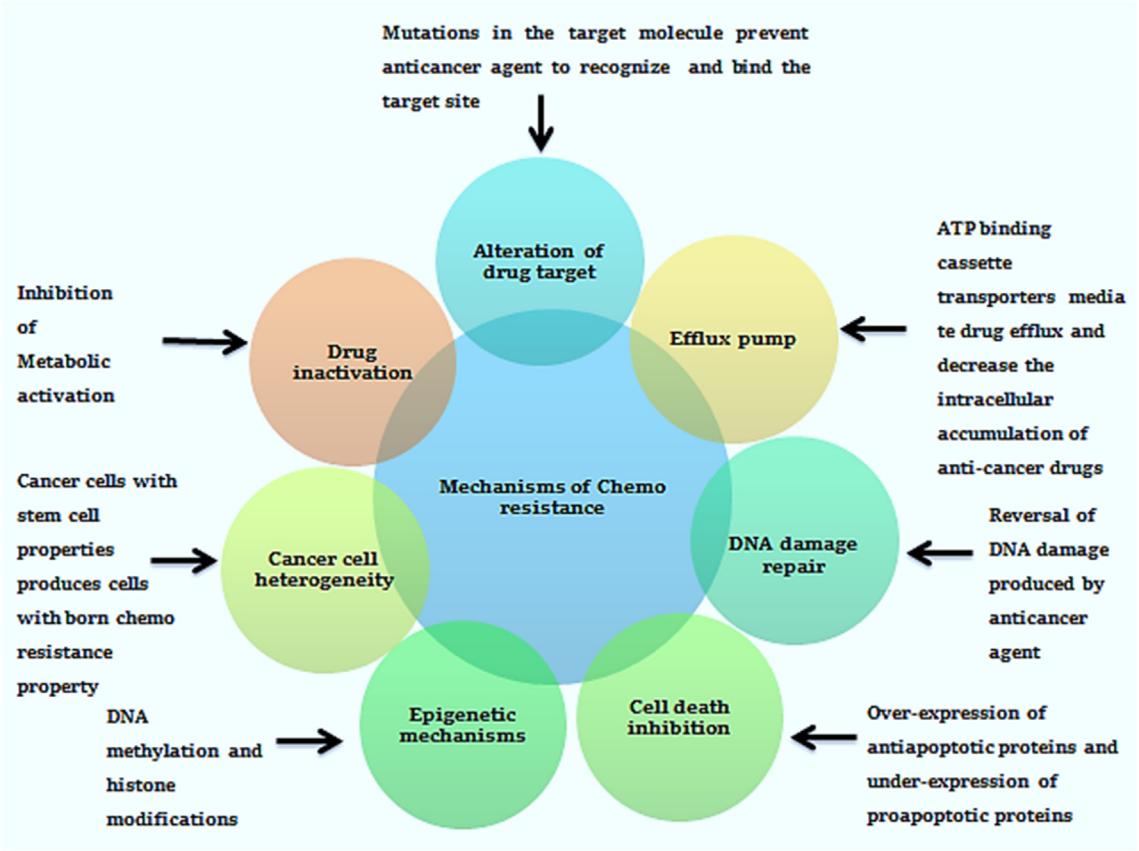

Figure 6 Mechanisms involved in chemoresistance.

The contribution of CSCs to therapeutic resistance and cancer metastasis

CSCs are involved in the prognosis of various tumor types and appear to be resistant to chemo and radiation therapy. The contribution of CSCs to drug resistance has been demonstrated in different cancer types, for example, pancreatic, colon, breast, and brain tumors.[110] Recently published articles emphasized CSCs and their implications in the generation of progenitor cells that involve the metastatic spread and relapse of cancer even after proper therapy and complete eradication of cancer cells. This distant site metastasis can be prevented by killing these progenitor cells through epigenetic drug treatment.[111] Further, understanding the CSCs' role in drug resistance facilitates developing better cancer therapy and preventing recurrence. Different underlying mechanisms for CSCs' contribution to therapeutic resistance and cancer metastasis was represented in Figure 7.

CSC microenvironment

CSCs can acclimatize and affect the tumor environment to facilitate a favorable environment for maintaining their stemness and enable self-renewal and differentiation, tumorigenesis, tumor invasion, and metastasis and, thus, sustain viability even after chemo and radiotherapy. CSC niche consists of diverse cell types such as endothelial cells, immune cells, cancer-associated fibroblasts, growth factors, and cytokines, making them maintain their stemness.[112] Hypoxia is another vital feature of the tumor environment and aids in the maintenance of stemness of CSCs, which promotes tumor survival and metastasis. The two hypoxia-inducible factors (HIFs), including HIF1α and HIF-2α, are overexpressed in response to hypoxic conditions and are related to tumor malignancy. HIF-2α activates the octamer-binding transcription factor 4 (Oct4), which is related to CSCs' self-renewal, and another transcription factor Sox2 is also by modulation of Oct4 levels in CSCs related to maintaining stemness. Hypoxia reduces reactive oxygen radicals' levels which is protective for CSCs, thus leading to tumor progression and therapy resistance. HIFs are believed to affect various pathways that contribute to the dormancy of CSCs by several means and are linked to multi-drug resistance, thus affecting drug efficacy.[113,114]

Multi-drug resistance

CSCs efficiently express ABC transporters including MDR1/ABCB1, MRP1/ABCC1, and ABCG2, which are multi-drug resistance proteins that protect solid tumor cells from being damaged by anti-cancer drugs and induce resistance. CSC markers, for example, ALDH, eliminate oxidative stress and enhance resistance to oxazolidine, taxanes, and

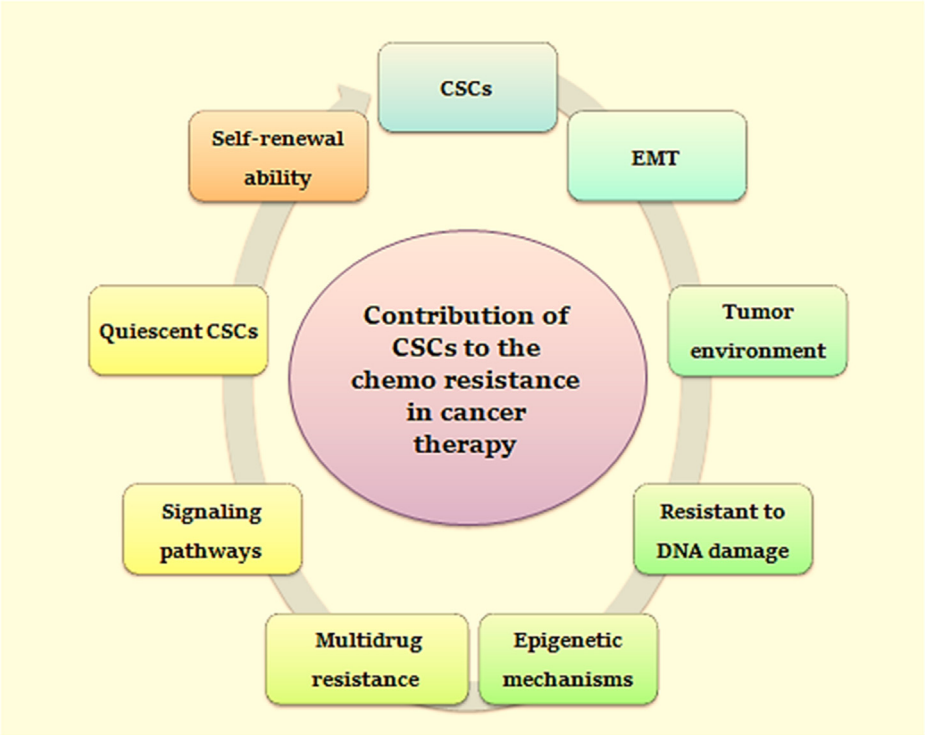

Figure 7 CSCs' contribution towards therapeutic resistance and metastasis.

platinum drugs. ALDH also brings resistance to radiation by removing radiation-induced free radicals.[115] Another common mechanism of chemo and radiotherapy is to induce DNA damage and apoptosis. However, CSCs can effectively escape from apoptosis by activating DNA repair mechanisms.

EMT

EMT is a complex process that occurs during normal embryonic development, tissue regeneration, organ fibrosis, and wound healing, where the epithelial cells are transformed into mesenchymal phenotype by losing their actual characteristics like cellular adhesion and cellular polarity. The tumor cells acquire stemness and accelerated migratory and invasive properties that play a key role in tumor progression and chemoresistance.[116,117] EMT can be driven by a large variety of developmental and growth factor signals by triggering genetic and epigenetic programs. These complex regulatory networks are under the control of EMT-transcription factors such as SNAI1/2, TWIST1, ZEB1/2, *etc*.[118] The known EMT inducers in cancer are hypoxia, cytokines, and growth factors secreted by the tumor microenvironment, stroma cross-talk, metabolic changes, innate and adaptive immune responses, and treatment with anti-cancer drugs. Also, the tumor cells present in multiple transitional states express mixed epithelial and mesenchymal genes with partial EMT, move collectively as clusters, and can be more aggressive than cells with a complete EMT phenotype. EMT is reversible, namely mesenchymal-to-epithelial transition (MET), likely affecting the circulating cancer cells when they reach a desirable metastatic niche and develop into secondary tumors.[119] Thus, the knowledge about the role and control of EMT in tumor progression, metastasis, and its reversion, is beneficial for improving anti-cancer therapy.

The level of CSCs differentiation and the degree of epithelial-mesenchymal transition defines the tumor metastatic grade. Depending on the metastatic grade, many factors are involved in developing drug resistance during EMT. For instance, drug resistance against Trastuzumab is developed in the tumor cells, which express high β1 integrin levels in ERBB2 (HER2) positive breast cancer.[120] Differentiation is necessary for the EMT, and drug resistance can also be developed during this signaling. Positive regulation of TGFβ by the overexpression of integrin αvβ1 in colon cancer cells, which is essential for EMT, can develop drug resistance.[121] Likewise, β3-integrin and Src regulate TGF-β mediated EMT in mammary epithelial cells.[122] Integrin β1-focal adhesion kinase signaling regulates the growth of metastatic cancer cells in the lungs.[123]

Possible approaches for targeting CSCs

The therapeutic elimination of CSCs thereby the complete eradication of tumors can be made possible by developing novel approaches to target different mechanisms involved in the CSCs' characteristics that contribute to the development of therapeutic resistance. Here, we discuss some important therapeutic approaches targeting CSCs through such underlying mechanisms.

Targeting CSCs regulating signaling pathways

Drugs targeting CSCs regulating signaling pathways are advantageous for better anti-cancer therapy. Psoralidin affected the Notch signaling pathway and inhibited CSC proliferation in the breast cancer model.[124] Cyclopamine, an Hdhg antagonist, depleted CSCs, induced tumor regression, decreased tumor growth rate, and inhibited CSC proliferation in different cancer types is currently being investigated as a treatment agent in basal cell carcinoma, medulloblastoma, and rhabdomyosarcoma glioblastoma, and as a treatment agent for multiple myeloma.[125] Vismodegib, a cyclopamine-derived drug, was approved by US FDA in 2012 for the treatment of basal-cell carcinoma and also clinically tested for other cancer types, including metastatic colorectal cancer, small-cell lung cancer, advanced stomach cancer, pancreatic cancer, medulloblastoma, and chondrosarcoma.[126] Both act as an antagonist for the smoothened receptor (SMO) and lead transcription factors GLI1 and GLI2 to remain inactive, preventing tumor-mediating gene expression within the Hdhg pathway. CD44, one of the CSCs markers, acts as a target for the Wnt signaling pathway regulating the self-renewal capabilities of CSCs and reducing tumor formation in an intestinal cancer model.[127] Targeting CSCs through the EMT pathways involves targeting the regulation of extracellular matrix components, hypoxia, transcription factors, and epigenetic mechanisms. Quite a few miRNAs were reported as CSC-based therapeutic agents in regulating CSCs' properties and reducing cancer malignancy, including mir-22, mir-200, Let-7, *etc.* in breast cancer, mir-128 in brain cancer, and other miRNAs in colon cancer and prostate cancer.[128]

Targeting surface markers

CD44 is overexpressed in several types of CSC populations and is involved in stemness and drug resistance. Aldehyde dehydrogenases (ALDHs) play important roles in cellular detoxification. High ALDH activity is found to enhance self-renewal capacity in breast and brain cancers and is known to confer chemoresistance to alkylating agents such as cyclophosphamide in hematopoietic and leukemic stem cells. Other membrane markers like CD34, CD38, CD123, TIM3, CD25, CD32, and CD96 are present on leukemia stem cells and are accountable for the chemotherapeutic resistance and disease relapse in acute myeloid leukemia.[129] CD117 is a transmembrane receptor involved in the stemness and differentiation of hematopoietic stem cells. $CD117^{+}$ cells can maintain high tumorigenic potential and differentiate into $CD117^{+}$ and $CD117^{-}$ cells in ovarian cancer.[130] As most of the CSCs markers are found on both normal cells as well as CSCs, specific antibodies are investigated. For example, anti-CD44 antibodies have shown their potential against different CSCs in pre-clinical investigations. CD133, another CSC marker used for identifying CSC populations in various solid tumors, and bi-specific antibodies that can isolate $CD133^{+}$ cells are developed.[131] Along with surface markers, the transcription factors such as NF-κB, HIF-1α, and β-catenin were also considered therapeutic targets to eradicate CSCs.[132]

Targeting ABC transporters

Targeting ABC transporters re-sensitizes resistance developed by CSCs to chemotherapeutics. Vardenafil improved the cytotoxicity of paclitaxel and vincristine by increasing their intracellular concentration by blocking P-gp-mediated drug efflux.[133] siRNA by targeting P-gp reversed drug resistance in a doxorubicin-resistant breast cancer model.[134] Superior CSC-targeting effects can be achieved by employing antibodies such as CD44 and EpCAM against CSC-specific biomarkers. These antibodies induce differentiation and inhibit proliferation in various cancer types.[135]

Enhancing immune responses

A more clinical benefit can be attained by using specific antibodies that specifically bind to the tumor-associated antigens (TAA). Several TAAs have been discovered, and their respective monoclonal antibodies were employed to target cancer cells. This modality can be considered a promising type of immunotherapy because the exact binding and expression of antibodies to the TAAs on target cells can be identified either by flow cytometry or immunostaining using the corresponding antibodies. Several immunological methods are in the research and development process. For instance, nonspecific anti-tumor immune activation involves enhancing the host immune system through nonspecific immunomodulation using FDA-approved cytokines such as IFNα and IL-2. IL-2 eliminates target cells by inducing T-cell expansion with major histocompatibility complex-specific recognition of TAA.[136] However, it can hinder the overall anti-tumor T cell function by up-regulating the $CD4^{+}$ CD25hi $Foxp3^{+}$ regulatory T cell (Treg) population and contributes to tumor immunoevasion. Adoptive transfer of chimeric antigen receptor (CAR) engineered T cells is another strategy with a high binding affinity to specifically target TAAs or cancer stromal antigens. For example, CAR T cell therapy inhibited tumor growth in highly metastatic prostate cancer types with low levels of EpCAM expression.[137] Table 2 lists a few antibodies that have been tried in research or clinical trials.

Targeting the CSC microenvironment

The heterogeneity of the CSC microenvironment, including cytokines, hypoxia, and perivascular niches regulating different CSC pathways and accounting for resistance against chemotherapy and radiation, serves as possible drug targets for CSCs. Repertaxin, a CXCR1/2 small-molecule inhibitor, decreased tumor volume and increased apoptosis of human gastric cancer MKN45 cells *in vitro* and *in vivo* and enhanced the efficacy of 5-fluorouracil.[148] Repertaxin treatment blocks IL-8/CXCR1 signaling and inhibits breast CSC self-renewal and survival.[149] Bortezomib, small molecule inhibitors of the HIF pathway approved by the FDA for multiple myeloma, and Temsirolimus for renal cell carcinoma, and most of the drugs such as methoxyestradiol, Echinomycin, Geldanamycin, *etc.*, were terminated either at phase I or II for not showing significant advantages in clinical trials.[150] Anti-vascular

Table 2 List of cancer cell markers (antibodies) used in immunotherapy for different cancers.

No.	Antibody	Cancer type
1	CD19	B-cell leukemias and lymphomas[138]
2	CD20	B-cell non-Hodgkin's lymphoma[139]
3	CD30	systemic anaplastic large-cell lymphoma[140]
4	CD33	Acute Myeloid Leukemia[141]
5	CD52	Chronic lymphocytic leukemia[142]
6	HER-2	Breast cancer[143]
7	EGFR	Colorectal or lung cancer[144]
8	VEGF	Colorectal cancer[145]
9	CEA	Gastrointestinal and many other cancers like medullary thyroid cancer, breast cancer, mucinous ovarian cancer, etc.[146]
10	EpCAM	Colorectal cancer[147]

endothelial growth factor agents, for example, Bevacizumab, Cediranib, Sunitinib, and Vandetanib are being tested in the initial phases of clinical therapy with moderate success.[151]

Nanoparticle-based delivery systems

The therapeutic efficacy of CSC-targeting agents is hindered by their hydrophobicity, poor specificity, and poor pharmacokinetic profiles. Nanoparticle delivery systems of CSC-targeted therapeutic agents were found to be more efficient and less toxic both *in vitro* as well as *in vivo* compared to free drugs. This is mainly due to controlled release kinetics, prolonged circulation time, and improved bio-distribution.[152] HPMA polymeric nanoparticles of a Hdhg pathway inhibitor efficiently targeted CSC populations and eliminated $CD133^+$ cells within prostate tumors.[153] Salinomycin therapeutic efficacy against $CD44^+$ drug-resistant cells was improved when conjugated with hyaluronic acid-based nanogel.[154] Nanoparticle delivery systems can incorporate multiple therapeutic agents in one carrier system allowing the co-delivery of cytotoxic drugs and CSC inhibitors that can target both bulk tumors and CSCs at once. Several RNAi molecules incorporated within the nanocarriers were found promising in CSC-targeted anticancer therapy as single or combinatorial immunotherapies; for example, targeting the Wnt pathway with nanoparticle-delivered siWNT1 halts tumor growth in a lung adenocarcinoma model and nanoparticle-delivered miR-34a inhibition of cell proliferation and migration of breast cancer.[155]

Combination therapy

Combination therapy has been evidenced as an important therapeutic strategy for treating cancer and other infectious diseases due to several advantages, such as enhanced efficacy in an additive or synergistic manner, less toxicity due to the possibility of dose reduction, and reversing/reducing drug resistance.[156] Combination therapy may include a neo-protector agent that protects normal cells or a repurposed agent usually intended either as an anti-cancer drug or another disease-related therapeutic and a secondary or tertiary anti-cancer agent that kills cancer cells and CSCs. Well-designed clinical research studies that test the combination of a repurposed therapeutic agent and another cytotoxic agent can reduce the financial burden associated with novel drug discovery as drug repositioning passes toxicity and safety profiles.[157] The combination of docetaxel and sulforaphane and pyrvinium pamoate inhibits the EMT, self-renewal ability of CSCs, and drug resistance by decreasing β-catenin expression in breast cancer.[158] The possible approaches for targeting CSCs are summarized in Figure 8.

Hurdles in new drug development for cancer therapy

Despite all hurdles, chemotherapy remains the most approachable and affordable strategy for cancer therapy. It is effective in reducing the cancer burden, easily accessible, and effortless to administer in patients of all stages of cancer.[159] However, the available anti-cancer drugs are limited in number and accompanied by severe side effects. Even though enormous attempts were being made in the development of new anti-cancer drugs, the disease remains one of the leading causes of mortality worldwide. Moreover, new technologies like structure-based drug discovery and molecular modeling techniques have been launched by pharmaceutical and biotechnological companies leading to increased expenditures on research and development.[160,161] Furthermore, adequate new safe drugs have not been delivered as per expectations. In addition, few drugs were found to be more expensive with less/low survival rates for cancer patients and are also associated with various side effects that made researchers search for alternate strategies.[162]

Life science researchers have developed a reasonable prognosis in therapeutic targets to bring new molecule entities into the market for cancer therapy. Due to the strict process of pre-clinical followed by clinical trials, the drug approval rate has declined since 1987.[162] In addition, the new drug development process involves a long time and procedures associated with huge costs and a high risk of failure. It is known that the process of drug administrative approval has been tedious; it takes around 10—15 years to complete all the formalities to bring drugs into the market and involves financial investment over billions of US dollars, approximately 12 billion US dollars.[163—165]

The traditional/conventional route of the new drug discovery process involves a phase-I study to confirm the maximum tolerated dose in humans, a phase-II study to investigate the pharmacodynamic and pharmacokinetic parameters for exploration of therapeutic benefit followed by a phase-III study comparing its efficacy to an established therapeutic or control in a larger population of volunteers and a phase-IV study evaluating the post-market adverse reactions and effectiveness in general population.[166] Moreover, in the approval process, there are many factors involved in the time and low success rate of new entity identification due to unproductive pre-clinical models,

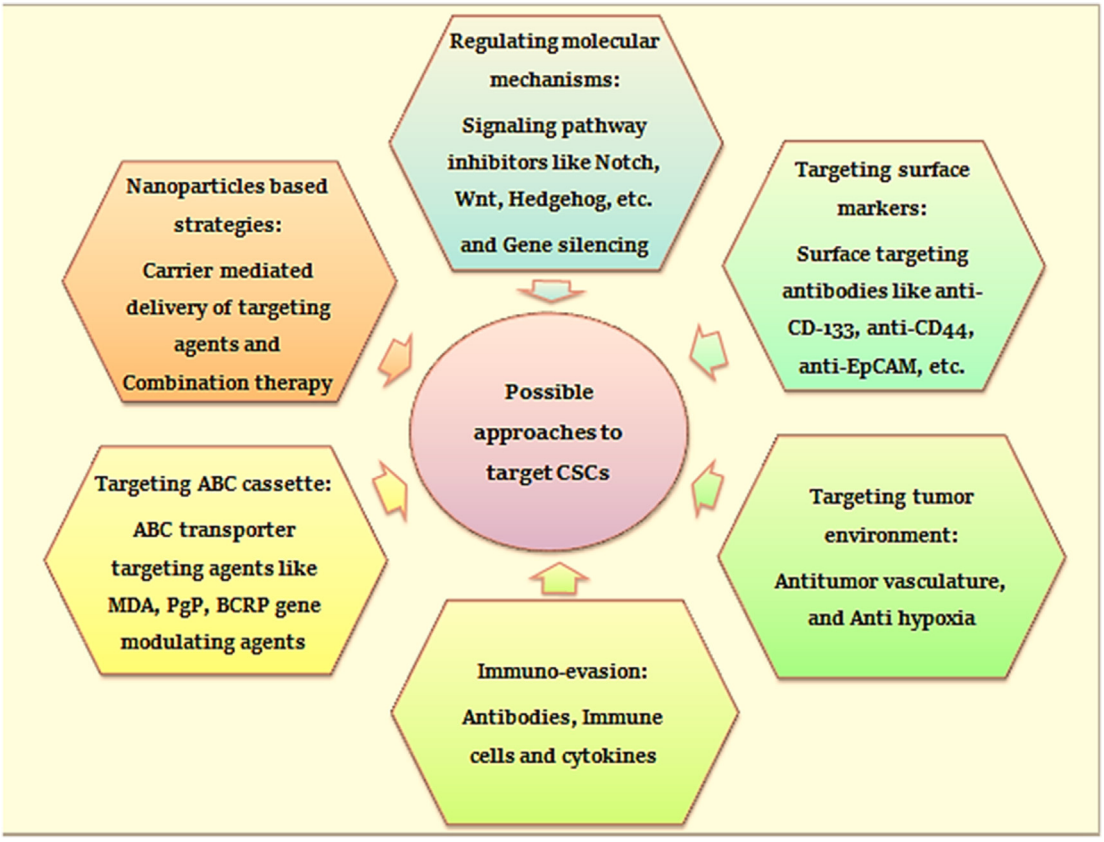

Figure 8 Possible approaches for targeting CSCs.

unexpected severe toxicity, unsuitable pharmacokinetics, *etc.*[167] It is a big stumbling block in reproducing pharmacological actions of animal data in human subjects due to age, gender, ethics, and other differences between human subjects and animals. This may be one of the main reasons that some drugs under pre-clinical investigation differ in their activities and fail in different phases of clinical trials.[168,169] Hence, there is a vast need to optimize the new strategy(s) to cut down the procedure and boost the success rate of existing drug development.

Drug repurposing

Drug repurposing or drug repositioning is a process of recognizing new pharmacological indications or therapeutic uses for drugs already approved and marketed for treating other diseases or therapeutic indications. Drug repurposing may be the best approach as diverse pre- and post-clinical trial data are available for these drugs with known toxicities and pharmacokinetic and pharmacodynamic behavior. Consequently, it resulted in making use of current non-cancer drugs for cancer therapy, thus reducing the expenditure for new drug discovery and development. It is estimated that 90% of approved non-cancer drugs, including anti-depressants, anti-convulsants, anti-hypertensives, statins, antihyperlipidemics, cardiac glycosides, antidiabetic, antiretroviral, antihelminthic, CNS drugs, anti-inflammatory, *etc.*, singly or in combination show positive effects, which may be employed for other usages (Table 3).

The strategy of drug repurposing

The strategy of drug repurposing works in either of two ways. One is that drugs like minoxidil may act on the same target and produces two different therapeutic effects. The other is that the drugs like aspirin may act on two individual targets to produce two different therapeutic actions when one target is known and the other is new/unknown (Fig. 9).[182]

Drug repurposing in cancer therapy

In recent years, drug repurposing in cancer research has emerged as an effective alternative to meet the increasing demand for new anti-cancer drugs. Itraconazole, an antifungal agent, acts as an SMO antagonist to suppress the growth of medulloblastoma in mice allograft models. Niclosamide, an anti-helminthic agent, decreased the level of CSCs by reducing the expression of LRP6 and β-catenin in basal-like breast cancer, reduced the expression of many components in the Wnt/β-catenin signaling pathway, the self-renewal ability, and population of CSCs of colorectal cancer.[183] Trifluoperazine, an anti-psychotic and anti-emetic agent, inhibits the spheroid formation ability of CSCs and suppresses CD44/CD133 markers' expression in lung CSCs by inhibiting Wnt/β-catenin signal transduction.[184] Actinomycin D and telmisartan lessen the number and activity of CSCs and reduce the expression of CSC markers ALDH1, SOX2, and NOS2 in lung cancer by

Table 3 List of approved non-cancer drugs for other therapeutic indications.

No.	Drug name	Approved indication/Category	Repurposed as	Mechanism/pharmacological action
1	Aspirin[170,171]	NSAID in the treatment of various pain and inflammatory disorders	An antiplatelet drug in the treatment of heart attacks and strokes; in the treatment of prostate cancer has also been reported.	Suppresses blood coagulation by inhibiting the normal functioning of platelets; inhibits overexpressed (COX-1/2) in prostate cancer cells.
2	Azithromycin[172]	An antibacterial antibiotic for bacterial infections	COVID-19	Azithromycin influences intracellular mitogen-activated protein kinase (MAPK), in particular, extracellular signal-regulated kinases 1/2 (ERK1/2) and the NF-κB pathway downstream of ERK.
3	Bupropion[173]	Anti-depressant to treat depression	Smoking cessation	Dopamine and noradrenalin reuptake inhibition with little serotonergic effect; also attenuates the stimulant effects of nicotine on the nicotinic acetylcholine receptors.
4	Chloroquine[174]	Anti-malarial malaria	COVID-19	The interference in the endocytic pathway, blockade of sialic acid receptors, restriction of pH-mediated spike (S) protein cleavage at the angiotensin-converting enzyme 2 (ACE2) binding site, and prevention of cytokine storm
5	Chlorpromazine[175]	Originally synthesized as an antimalarial	Used as sedative-anxiolytic in acute mania as an adjunct to barbiturates and later also for Covid-19	Inhibit clathrin-mediated endocytosis
6	Colchicine[176]	An anti-inflammatory agent used in the treatment of gout/gouty arthritis	Pericarditis/COVID-19	Reducing the chemotaxis of neutrophils, inhibiting inflammasome signaling, and decreasing the production of cytokines, such as interleukin-1 beta
7	Dexrazoxane[177]	Antimitotic	Cotreatment to prevent anthracycline-induced extravasation injuries81 and cardiomyopathy, cotreatment is also used for immunosuppressive purposes.	Dexrazone's effect may be due to its ability to inhibit the formation of a toxic iron—anthracycline complex.
8	Dimethyl fumarate,[178]	Anti-allergic psoriasis, multiple sclerosis	Atherosclerotic cardiovascular disease	Activation of Nrf2, Inhibition of NF-κB, agonism of HCAR2, inhibition of aerobic glycolysis, and depletion of GSH
9	Fluoxetine[179]	Anti-depressant	Depression	Premenstrual dysphoria
10	Minoxidil[180]	Antihypertensive vasodilator	Used as an anti-hair loss drug in the treatment of male pattern baldness (androgenic alopecia)	Minoxidil widens blood vessels and opens potassium channels, allowing more oxygen, blood, and nutrients to the hair follicles.
11	Remdesivir[181]	Antiretroviral to treat HIV/AIDS	COVID-19	Inhibit RdRp/viral replication inhibitor

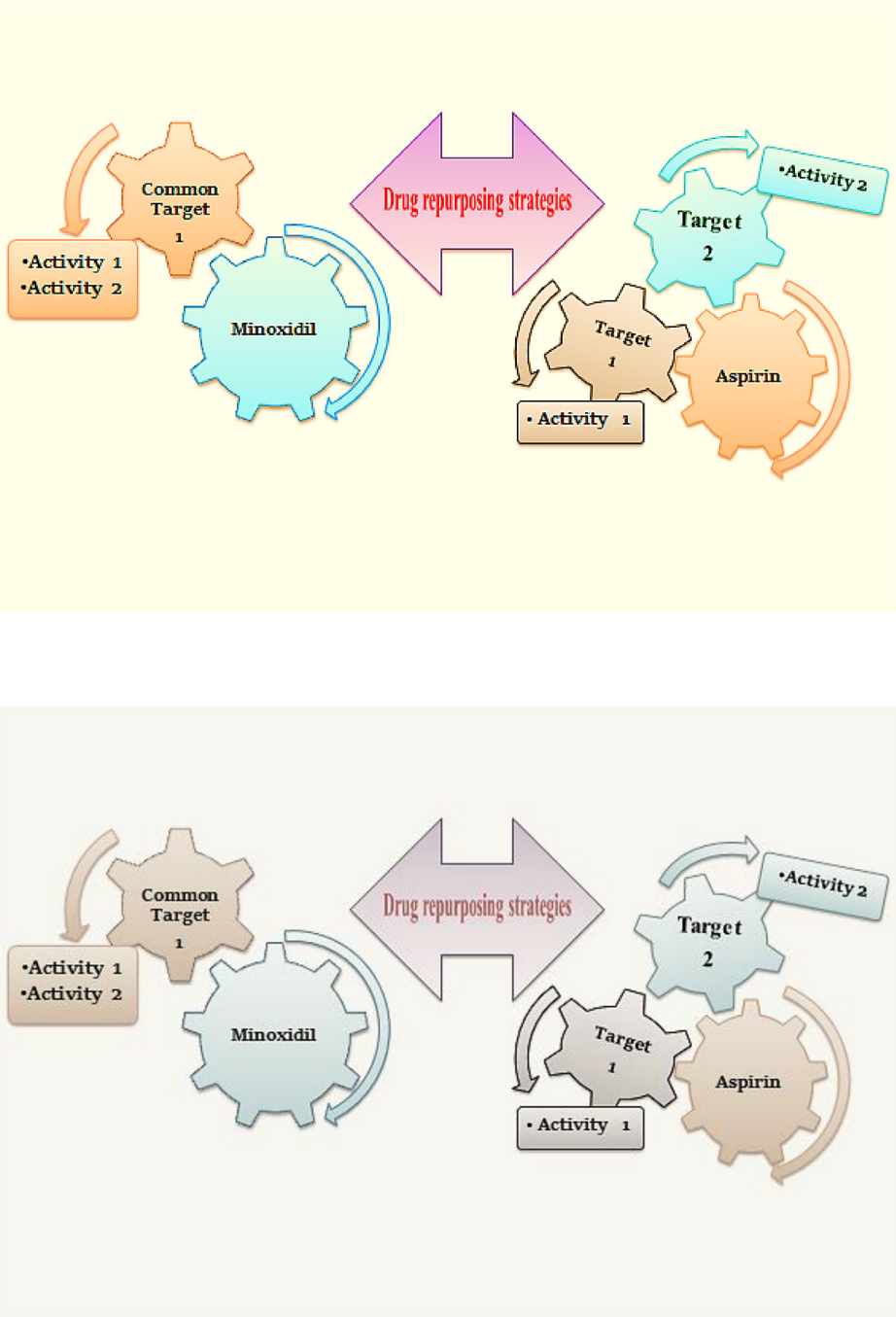

Figure 9 Drug repurposing strategies: Minoxidil binds with a single target to produce both activities 1 and 2, whereas aspirin binds with Target 1 to produce activity 1 and Target 2 to produce activity 2.

blocking the Wnt/β-catenin signaling pathway.[185] Table 4 consists of some of the known non-cancer drugs targeting CSCs for therapy.

There is less risk of failure for the existing non-cancer drugs in approval when we compare the approval of new anti-cancer drugs through the new drug discovery process. This is because the existing non-cancer drugs have available pre-clinical (pharmacological, toxicological, *etc.*) as well as clinical efficacy and safety information as they underwent the early stages of drug development for other therapeutic uses. We have listed out and compared the different steps involved and the pros and cons of the traditional new drug discovery and drug repurposing processes in Figure 10, which explains the merits of available non-cancer drugs for targeting CSCs *vs.* the synthesis of new anti-cancer drugs. Among different strategies adopted by clinicians as well as researchers to minimize the cost and time in the drug development process, drug repositioning, in other words, using old drugs for the new purpose of targeting cancer disease is attractive where it takes the benefit of already

Table 4 List of some of the known non-cancer drugs targeting CSCs for therapy.

No.	Drugs	Category	Targeting CSCs	Mechanism of action
1	Abamectin	Pesticide	Epithelial	Selectively restrict CSC growth[186]
2	Acyclovir	Anti-viral	breast cancer	Down-regulate ALDH activity in MCF7 cells[187]
3	All trans-retinoic acid	Acne vulgaris	Head and neck squamous, breast, acute leukemia	Induce CSCs differentiation by suppression of Wnt/β-catenin pathway, blocks multiple Pin1-regulated cancer-driving pathways[188]
4	Aripiprazole	Anti-psychotic	Colon, glioma, and gastric cancer	slows down the proliferation of cells and tumor growth[189]
5	Artemisinin	Anti-malarial	Colon, breast, ovarian, prostate, pancreatic, and leukemia cancer cells	Multiple potential mechanisms include anti-proliferative effects through cell-cycle disruption (ROS)-induced DNA damage, induction of apoptosis, anti-angiogenesis, immunomodulation, and induced radiosensitivity.[190]
6	Aspirin	Anti-pyritic and anti-inflammatory	Pancreatic, prostate, colorectal, breast, lung, and skin cancer	Induce apoptosis and interfere in cell signaling[191,192]
7	Brexpiprazole	Anti-psychotic	Pancreatic, non-small cell	Affect the self-renewal ability of CSCs by reducing the sphere formation ability of CSCs[193]
8	Brivudine	Anti-viral	Pancreatic cancer	Showed anti-cancer properties by suppressing the chemoresistance[194]
9	Cimetidine	H2 receptor antagonist in the treatment of gastric ulcer peptic ulcers	Breast, cervical, lung, ovarian, colorectal, prostate, gastric tumors, and gliomas	Affect cell proliferation, immunomodulation, cell adhesion, and angiogenesis through different mechanisms[195]
10	Clarithromycin	Anti-bacterial	Lung cancer	Cause break in the DNA by intercalation and thereby inhibiting DNA replication[196]
11	Chloroquine	Anti-malarial	Triple-negative breast cancer	Alter the Jak2-STAT3 pathway and DNMT1 expression in addition to autophagy inhibition[197]
12	Curcumin	Anti-oxidant, anti-inflammatory	Multiple myeloma, breast, colon, and prostate	Suppression of cytokines release, particularly interleukin (IL)-6, IL-8, and IL-1, which stimulate CSCs, and also alter its effects at multiple sites along CSC pathways such as Wnt, Notch, Hdhg, and FAK signaling pathways[198]
13	Cyclopamine	Steroidal alkaloid	Basal cell carcinoma	Bind to the Smo protein to inhibit the Hh pathway[199]
14	Diclofenac	Anti-inflammatory	Colorectal, breast, pancreatic, ovarian, and prostate cancer	Immunomodulation, apoptosis, angiogenesis, actions on Myc, and glucose metabolism[200]
15	Disulfiram	Anti-alcoholism	Breast	Inhibit TGF-β-induced EMT and CSC-like features in breast cancer cells[201]
16	Doxycycline	Anti-bacterial	Breast	Inhibit the stem cell phenotype of cancer cells as well as the process of mitochondrial biogenesis[202]
17	Epigallocatechin gallate	Dietary supplement	Breast, colorectal, head, and neck	Self-renewal regulation via Notch, Wnt, and polycomb repressive complexes pathway[203]
18	Genistein	Anti-oxidant	Prostate	Inhibit Hh pathway[204]
19	Ginsenoside F2	Reduce hair loss	Breast	Induce apoptosis in breast CSCs by activating the intrinsic apoptotic pathway and mitochondrial dysfunction[205]

(*continued on next page*)

Table 4 (*continued*)

No.	Drugs	Category	Targeting CSCs	Mechanism of action
20	Hydralazine	Anti-hypertensive	Metastatic cervical and ovary cancer (phase III clinical trial), and breast cancer (phase II clinical trial) to overcome chemotherapy resistance.	Ability to demethylate and reactivate tumor suppressor gene expression; activates the intrinsic pathway of apoptosis and causes DNA damage in leukemic T cells.[206,207]
21	Ibuprofen	Analgesics/antipyretics	Gastric cancer	Reduce cell proliferation through inhibiting Wnt/β catenin signaling pathway[208]
22	Indomethacin	Anti-inflammatory	Colorectal	Inhibit cyclooxygenase and NOTCH/HES1 and activate peroxisome proliferator-activated receptor γ (PPARG)[209]
23	Itraconazole	Anti-fungal	Human umbilical vein endothelial cells, endometrial carcinoma, melanoma cells, and glioblastoma.	Inhibit AKT/mTOR signaling pathway; reverses chemoresistance induced by P-glycoprotein, regulating the signal transduction pathways of Hdhg, inhibiting angiogenesis and lymphangiogenesis of cancer cells.[210]
24	Mebendazole	Anti-fungal	Non-small cell lung cancer, breast cancer cells	Tubulin depolymerization induced cell cycle arrest; reduces the proportion of stem cells through the Hdhg pathway to respond to DNA damage.[211]
25	Mesalazine	Anti-inflammatory	Colorectal cancer, ulcerative colitis	Inhibits Wnt/Beta-catenin pathway[212]
26	Metformin	Antidiabetic	Breast, prostate, lung, endometrial and pancreas, ovarian cancer, osteosarcoma	Inhibits CSCs' self-renewal ability and AMPK/mTOR independent pathways, reactive oxygen species-mediated apoptosis, and autophagy[213–215]
27	Thiazolidinediones	Diabetes	Prostate	Cell proliferation and apoptosis through interaction with the insulin-like growth factor-I receptor/AKT/mTOR signaling pathway[216]
28	Niclosamide	Antihelminthic	Colorectal, breast, ovarian cancer, and other malignancies	Multiple signaling pathways, such as Wnt/β-catenin, STAT3, and NF-κB, contribute to cancer invasion and metastasis[217]
29	Nigericin	Antibiotic	Ovarian	Selectively target CSCs[218]
30	Phenformin	Diabetic	Melanoma	Inhibit transformation[219]
31	Piperine	Immunomodulatory, anti-oxidant	Lung, breast	Restrict the self revival[220]
32	Quinacrine	Rheumatic disease	Breast	Induce apoptosis and inhibit the Hh pathway[221]
33	Raloxifene	Osteoporosis	Invasive breast cancer	Inhibit proliferation[222]
34	Rapamycin	Immunosuppressant	Approved to treat renal cancer; in clinical trials to treat various other cancers	Inhibit tumor growth by halting tumor cell proliferation, inducing tumor cell apoptosis, and suppressing tumor angiogenesis; Rapamycin allosterically inhibits mTORC1 by binding to the FRB domain of mTOR[223]
35	Ritonavir	Anti-viral	Pancreatic cancer, lung cancer, breast cancer, renal cancer cells, multiple myeloma, ovarian malignant growth cells, *etc.*	Ritonavir has been shown to decrease cancer cell growth and division, accelerate apoptosis endoplasmic reticulum stress and hinder numerous signaling pathways like AKT and nuclear factor-kappa B pathways[224]
36	Sabutoclax	New antibiotics	Leukemia	Inhibit BCL2 family proteins[225]
37	Salinomycin	Antibiotic	Breast	Inhibit WNT and modulate Hh signaling pathways[226]
38	Sertindole	Anti-psychotic	Breast and gastric cancers	Anti-proliferative activities by inhibiting the STAT3 signaling pathway[227]

39	Simvastatin	Statin/lipid-lowering agent	C6 glioma cells	JNK-dependent activation of ATF-2 and c-jun, reduced cell proliferation, and induced apoptosis[228]
40	Soy Isoflavone	Fertility	Breast	Inhibit proliferation and Wnt pathway[229]
41	Sulforaphane	Autism	Breast	Act on miR-124/IL-6R/STAT3 axis[230]
42	Thioridazine	Anti-psychotic	Leukemia	Induced autophagy[231]

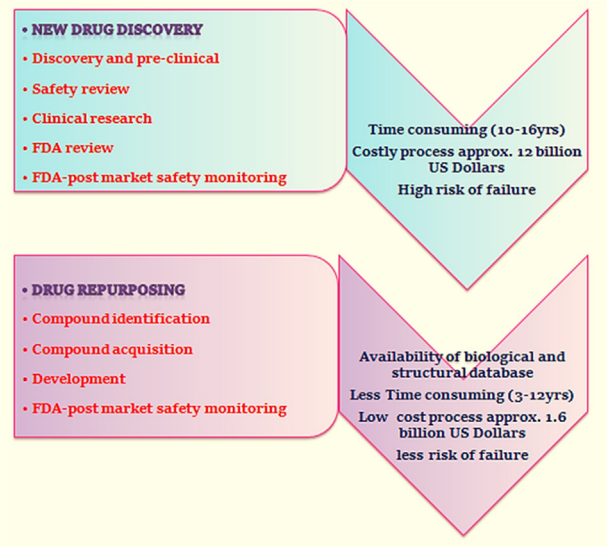

Figure 10 Differences between new drug discovery and drug repurposing.

existing, well-characterized, and widely-used, non-cancer drugs for testing them as successful anti-cancer agents.[232] Here, we suggest an easier and safe model in which one can select existing non-cancer drugs that are available in the market and test for their anti-cancer activity with special emphasis on targeting CSCs.

Clinical studies on drug repurposing for cancer therapy

The efficacy of anti-CSC agents is being investigated in clinical trials focusing on reduction in tumor volume, progression-free, and survival endpoints. The Anticancer Fund is an independent, non-profit research organization involved in investigator-driven clinical trials to bring new treatment options for patients as speedily as possible. They mainly explore phase II and III trials to investigate and confirm the therapeutic efficacy of repurposed drugs and combination therapy. A French study, Fluvabrex for children with brain cancer, tested a combination of two non-cancer drugs, one an anti-cholesterol and the other an anti-inflammatory, to see if this less toxic treatment was active against low-grade gliomas in children. The tumor progression was suppressed for more than or equal to 6 months in 7 of the 10 children and for more than or equal to 3 years in 3 of them. Few more studies are being performed to investigate new treatment options using repurposed drugs in different types of cancer, which would require a more toxic treatment based on chemo and radiotherapy (Table 5).[233]

Phytonutrients in cancer therapy

Several phytonutrients are being demonstrated as promising sources of anti-cancer agents by affecting the regulation of various signaling pathways, together with autophagy and apoptosis.[234] Sulforaphane, resveratrol, lycopene, epigallocatechin, curcumin, and berberine are

Table 5 Clinical studies using different repurposed drugs for cancer therapy.

No.	Study title	Study objective	Cancer type	Trial phase	Status	Outcome
1	Anti-inflammatory and cholesterol inhibitors as repurposed drugs for children's optic nerve cancer	A phase I study assesses the safety of the association of fluvastatin and celecoxib in children with refractory optic-pathway glioma (Fluvabrex).	Pediatric brain cancer	1	Completed	The combination of the two drugs displayed very limited toxicity with interesting preliminary activity in low-grade gliomas
2	Combination of 3 repurposed drugs after chemotherapy failure in lung cancer	A prospective phase II, randomized multi-center trial of a bio-modulatory treatment with metronomic low-dose treosulfan, pioglitazone, and clarithromycin versus nivolumab in patients with squamous cell lung cancer and non-squamous cell lung cancer, respectively, after platin failure (ModuLung).	Lung cancer: Clarithromycin - antibiotic, Pioglitazone - anti-diabetes, Treosulfan - a chemotherapeutic drug used at a metronomic dose.	2	Terminated	In other words, the new treatment did not improve the outcomes of the patients compared to the standard treatment.
3	Repurposing of Decitabine in Kras-Dependent Refractory Pancreatic Cancer	A proof-of-concept, biomarker-driven, phase-II clinical trial to explore the repurposing of decitabine against advanced, refractory, KRAS-dependent pancreatic ductal adenocarcinoma (PDAC): The ORIENTATE trial.	Digestive cancer	2	Recruiting	—
4	Neoadjuvant β-blocker in angiosarcoma patients	Neoadjuvant trial on the efficacy of propranolol monotherapy in patients with angiosarcoma (PropAngio).	Sarcoma	2	Recruiting	—
5	Vitamin D treatment for melanoma	This is a phase III, double-blind, randomized, placebo-controlled trial of high-dose vitamin D3 (cholecalciferol) in patients with stage IB to III cutaneous melanoma.	Melanoma	3	Recruiting	—
6	Aspirin for recurrence and survival in colon cancer	A phase III double-blind placebo-controlled randomized trial of aspirin on recurrence and survival in colon cancer patients (ASPIRIN).	Digestive cancer	3	Recruiting	—
7	Curcumin in endometrial cancer	This was a monocentric, prospective phase II trial to determine the capacity of curcumin to reduce inflammatory mediators and immunomodulatory cell types in endometrial carcinoma.	Gynecological cancer	3	Completed	Curcumin phytosome - induced immunomodulatory effects in endometrial carcinoma were modest without significant quality of life changes.

8	Repurposing angina pectoris medication as lung cancer treatment	Single center non-randomized phase II trial To study the effect of nitroglycerin in non-small cell lung cancer on survival	Lung cancer	2	Completed	Adding nitroglycerin to radiotherapy in non-small cell lung cancer did not improve survival compared to what would have been expected without nitroglycerin.
9	Advanced bone cancer treatment with a combination of chemotherapy and immunosuppressants	A phase Ib study of metronomic cyclophosphamide and methotrexate combined with zoledronic acid and sirolimus in patients with solid tumors with bone metastasis and advanced pretreated osteosarcoma (Metzolimos).	Solid tumors Bone cancer	1 b	Completed	The dose of sirolimus that can be safely administered in combination with the 3 other drugs was determined.
10	Combination of 9 repurposed drugs with low-dose chemotherapy for brain cancer	Monocentric proof of concept clinical trial assessing the safety of the coordinated undermining of survival paths by 9 repurposed drugs (aprepitant, auranofin, celecoxib, captopril, disulfiram, itraconazole, minocycline, ritonavir, and sertraline) combined with metronomic temozolomide for recurrent glioblastoma (CUSP9v3).	Brain cancer	1	Completed	Ritonavir, temozolomide, captopril, and itraconazole were the drugs most frequently requiring dose modification or pausing. Progression-free survival at 12 months was 50%.
11	Perioperative anti-inflammatory to reduce breast cancer recurrence	This is a prospective, randomized, placebo-controlled, double-blind study with an inclusion period of 2 years and a 5-year follow-up period to determine if perioperative administration of ketorolac during breast cancer surgery affects survival rates.	Breast cancer	3	Completed	A single injection of ketorolac - an NSAID - just before breast cancer surgery does not improve disease-free survival.
12	Treating leukemia patients with chemotherapy and 2 repurposed drugs	A randomized, phase II, open-label, multi-center with safety run-in phase evaluating low-dose azacitidine, all-trans retinoic acid (ATRA), and pioglitazone versus standard dose azacitidine in patients $\geq$60 years of age with acute myeloid leukemia (AML) who are refractory to standard induction chemotherapy (AML-ViVA).	Blood cancer	2	Completed	The treatment is safe in this very fragile population, and 3 out of 10 patients had a complete response. One additional patient had stabilization of his disease for 14 months.

(*continued on next page*)

Table 5 (*continued*)

No.	Study title	Study objective	Cancer type	Trial phase	Status	Outcome
13	Antimalarial drug repurposed as a treatment for liver cancer	A single-center phase I dose-escalation study evaluating the safety and pharmacokinetics of oral artesunate (ART) in patients with advanced hepatocellular carcinoma (HCC).	Liver cancer	1	Terminated	–
14	Sodium bicarbonate for tumor-related pain	This is a single-institution, non-randomized phase I/II study of sodium bicarbonate in combination with standard medical management, a stable opioid regimen, for 35 patients with moderate to severe tumor-related pain due to solid metastatic malignancies or hematologic malignancies.	Blood cancer, solid tumors	1/2	Terminated	Patients could hardly comply with the treatment protocol because of the poor tolerability of $NaHCO_3$, and the project was prematurely closed.

Source: https://www.anticancerfund.org/en/drug-repurposing.

some phytochemicals that exerted their anti-cancer potential *in vitro* and *in vivo* through modulating the autophagy-apoptosis in clinical trials for different cancer types.[235] Furthermore, dietary components such as capsaicin, cucurbitacin B, isoflavones, catechins, lycopene, benzyl isothiocyanate, phenethyl isothiocyanate, and piperlongumine have demonstrated inhibitory effects on cancer cells, indicating that they may serve as chemopreventive agents. They inhibit the development of cancer either by impeding DNA damage, which leads to malignancy or by reversing or blocking the division of premalignant cells with DNA damage.[236] Clinically tested phytochemicals, Allium sativaum, camptothecin, curcumin, green tea, *etc.*, reduced cancer progression in cancer patients. Allium sativum increased the number and activity of natural killer cells in colorectal, liver, and pancreatic cancer.[236,237] Piper longumine caused autophagy-mediated apoptosis by inhibiting PIK3/Akt/mTOR in multiple myeloma, melanoma, pancreatic cancer, colon, breast, and prostate cancer.[238] Capsaicin acts by several means; for example, it blocks AP1, NF-kB, and STAT3 signaling, cell cycle arrest, and inhibition of β-catenin signaling in pancreatic cancer.[239,240] Catechins obtained from green tea showed cell cycle arrest at the G2 phase and protection against oxidative stress affecting STAT3-NF-kB and PI3k/Akt/mTOR in neuroblastoma, breast, and prostate cancer.[241]

Combination of phytonutrients with anti-cancer drugs

The combination of anti-tumor phytochemicals can be more effective in modulating different signaling pathways associated with tumor cell growth which is the common target for anti-tumor action. Piperine, in combination with Paclitaxel, showed a synergistic anti-cancer effect *in vitro* in the MCF-7 cell line[242] and reduced the IC50 value of paclitaxel *in vitro* in the MDA MB-231 cell line.[243] Thymoquinone in combination with Gemcitabine synergizes the apoptotic activity *in vitro* in MCF-7 and T47D cell lines.[244] Sulforaphane with Paclitaxel enhances paclitaxel-induced apoptosis *in vitro* in MDA-MB-231 and MCF-7 cell lines, and with Doxorubicin showed better tumor regression and even eradicated the tumors in all rats by day 35 after tumor implantation *in vivo* in rat orthotopic breast cancer model (inoculated with MAT B III).[245] Thymoquinone with Paclitaxel sensitized 4T1 cells to paclitaxel and induced higher cytotoxicity and with Gemcitabine, synergized the apoptotic activity in MCF-7 and T47D cell lines.[246,247]

Combination of repurposed drugs with micronutrients for a superior approach

From recent investigations, CSCs are considered the key reason behind the cancer disease progression and development of all types of tumors. These CSC cells are often isolated from different cancers of the brain, blood (leukemia), skin (melanoma), breast, head and neck, thyroid, cervix, lung, organs of the gastrointestinal and reproductive tract, and retina.[248] A successful therapeutic approach may be believed to eliminate the CSC population from the

tumors. Chemotherapy, radiation, and immunotherapy are found to be the popular conventional anti-cancer therapies that can reduce the tumor mass by killing the rapidly growing cells of tumors but potentially leave behind the cancer-initiating cells. The multi-drug resistance transporter1 (MDR1) and ABCG2 can facilitate the efflux of DNA binding dyes such as Hoechst 33342 in cells with cancer stem cell activity known as the side population. The ability of multi-drug resistance in CSCs led to the efflux of anti-cancer drugs such as doxorubicin, gemcitabine, and mitoxantrone. Moreover, in the presence of mitoxantrone, the SP cell frequency was increased in neuroblastoma. Enhanced resistance of $CD133^{+}$ brain tumor stem cells to chemotherapeutic agents such as paclitaxel, carboplatin, etoposide, and temozolomide have been attributed to higher expression of ABCG2, increased activity of DNA mismatch repair genes, and an altered balance of intracellular pro-/anti-apoptotic factors.[249]

Micronutrients have been explored for cancer therapy due to their negligible or no side effects and are adequately available in food sources. But sufficient data is not available for therapeutic exploration. There is certain evidence that micronutrients such as vitamins, for example, all-transretinoic acid (ATRA), vitamin C, vitamin D, *etc.*, have been tested for anti-cancer activity in a variety of cancers where they showed significant anti-cancer activity with or without combinations for other therapies.[250] ATRA has been tested in a variety of blood-related cancers, including myelodysplastic syndromes, multiple myeloma, and chronic myelogenous cancers.[251] Retinoic acid was also studied in different tumors, including breast cancer, prostate, and glioma cancers. Apart from this, ATRA is also a well-known proliferation inhibitor and differentiation inducer of malignant cells.[252]

The popular micronutrient vitamin B6 has been shown to have an effective role in significantly reducing different cancer risks such as colorectal, pancreatic, gastric adenocarcinoma, oral, lung, prostate, and breast cancer.[253—258] Many *in vitro* studies revealed that vitamin C exhibits selective toxicity towards malignant melanoma cells, human leukemia cells, neuroblastoma cells, tumor ascites cells, as well as acute lymphoblastic leukemia, epidermoid carcinoma, and fibrosarcoma, where it acted as a pro-oxidant in different cancer cell lines, and recent report emphasized that higher concentration of vitamin C has 1000-fold more anti-cancer activity with a special focus of targeting CSCs.[259,260] Vitamin D is known to be a potential therapeutic agent in anti-cancer therapy. However, the possible actions of vitamin D systems implicated in cancer development may be exerted upon specific histologic subtypes of cancer, mainly breast, prostate, and colorectal cancer cells. Vitamin D and its analogs have been shown inhibitory effects on the cancer stem cell signaling pathways, suggesting vitamin D may serve as a potential preventive or therapeutic agent against CSCs.[261]

Anti-cancer properties of these micronutrients may target cancer cells, and drugs like metformin, thioridazine, salinomycin, *etc.*, are recently popular in targeting CSCs. The combination of cancer and CSC-targeted drugs may be useful to restrict cancer as well as CSC growth at a single time point, which is believed to be an effective treatment strategy at present.

Conclusions

CSCs play key roles in tumor progression, metastasis, and cancer recurrence due to their ability to develop chemo- and radio-resistance. Thus, targeting CSCs became necessary to diminish tumor prognosis for better cancer therapy. Selective CSC targeting can be achieved by using anti-cancer agents that can interfere with different signaling pathways that are essential for their stem cell properties and tumor microenvironment which is a challenge in the development of new cancer treatment strategies. However, the combination therapy that includes conventional therapy and targeted therapy against CSC-specific pathways, as well as mechanisms contributing to drug resistance to chemotherapy and radiation, may provide complete removal of both bulk tumors as well as the CSC population. As the new drug development process involves long time procedures associated with huge costs and a high risk of failure, we suggest drug repurposing of available non-cancer drugs as the easier and safe model to test for their anti-cancer activity with special emphasis on targeting to CSCs and underlying mechanisms contributing to therapeutic resistance.

Author contributions

Dr. Rajanna Ajumeera: Conceptualization, design, and supervision.
Dr. Divya Ajmeera: Manuscript writing and editing, preparation of figures and tables, proof reading and comunication.

Conflict of interests

The authors have no competing interests to declare.

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Appendix A. Supplementary data

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