Targeting Unique Features of Quiescent Cancer Stem Cells to Overcome Resistance andRecurrence in Cancer Therapy: Systematic Review

Abstract

Quiescent cancer stem cells are non-proliferating cells arrested in the G0 phase, characterized by low ki-67 expression and high p27 expression. QCC avoids most chemotherapy, and some subsequent treatments may result in a higher proportion of QCC in the tumor. QCCs are also associated with cancer recurrence because they can re-enter a proliferative state when conditions are favorable. Because QCC causes drug resistance and tumor recurrence, there is a great need to understand the characteristics of QCC, decipher the mechanisms that regulate the proliferative-quiescent transition in cancer cells, and develop new strategies to eliminate QCC residing in solid tumors. In this review, we discuss the mechanisms of QCC-induced drug resistance and tumor recurrence. We also discuss therapeutic strategies to overcome resistance and recurrence by targeting QCC, including (i) identifying reactive quiescent cancer cells and eliminating them via cell cycle-dependent anticancer reagents; (ii) modulating the quiescence-to-proliferation switch; and (iii) eliminate QCC by targeting its unique features. Co-targeting proliferating and quiescent cancer cells may ultimately lead to the development of more effective therapeutic strategies for cancer treatment.

Keywords: quiescent cancer stem cells, targeted therapy

Introduction

Cancer is a chronic disease that really threatens human life. Many strategies have been developed in cancer treatment, including radiotherapy, chemotherapy, surgery and targeted therapy. The incidence of cancer in women has stabilized and decreased slightly in men in the last decade, and the death rate from cancer has also decreased [1]. However, traditional cancer treatment methods are only effective for some types of cancer [2]. The main reason for failure of cancer treatment is recurrence , metastasis, heterogeneity, resistance to chemotherapy and radiotherapy, and evasion of immunological surveillance [3]. All

these failures can be described by the characteristics of the cancer. cancer stem cells [4]. Cancer stem cells can cause cancer recurrence, metastasis, multidrug resistance, and radiation resistance through their ability to survive in the G0 phase, thereby giving rise to new tumors [5]. Therefore, cancer stem cells can be considered the most important target promising for cancer treatment.

Cancer stem cells were first identified in leukemia patients and then isolated through expression of surface markers CD34+ and CD38− in the 1990s [6,7]. Cancer stem cells expressing different surface markers, such as CD133, nestin, and CD44, were later discovered in many nonsolid tumors. and solid. These cells also make up the majority of tumors [8,9]. CSCs can generate tumors through self-renewal and differentiation into multiple cellular subtypes [10]. CSC activity is controlled by many intracellular and extracellular factors, and these factors can be used as drug targets for treatment cancer [11]. To understand the properties of CSCs, we summarize effective cancer stem cells-targeting therapeutic methods for cancer therapy in both basic research and clinical studies.

Characteristics of Quiescent Cancer Stem Cells

Quiescent cancer stem cells are non-proliferating cells arrested in the G0 phase, characterized by low ki-67 expression and high p27 expression. QCC avoids most chemotherapy, and some subsequent treatments may result in a higher proportion of QCC in the tumor. QCCs are also associated with cancer recurrence because they can re-enter a proliferative state when conditions are favorable. Because QCC causes drug resistance and tumor recurrence, there is a great need to understand the characteristics of QCC, decipher the mechanisms that regulate the proliferative-quiescent transition in cancer cells, and develop new strategies to eliminate QCC residing in solid tumors. In this review, we discuss the mechanisms of QCC-induced drug resistance and tumor recurrence. We also discuss therapeutic strategies to overcome resistance and recurrence by targeting QCC, including (i) identifying reactive quiescent cancer cells and eliminating them via cell cycle-dependent anticancer reagents; (ii) modulating the quiescence-to-proliferation switch; and (iii) eliminate QCC by targeting its unique features. Co-targeting proliferating and quiescent cancer cells may ultimately lead to the development of more effective therapeutic strategies for cancer treatment [12].

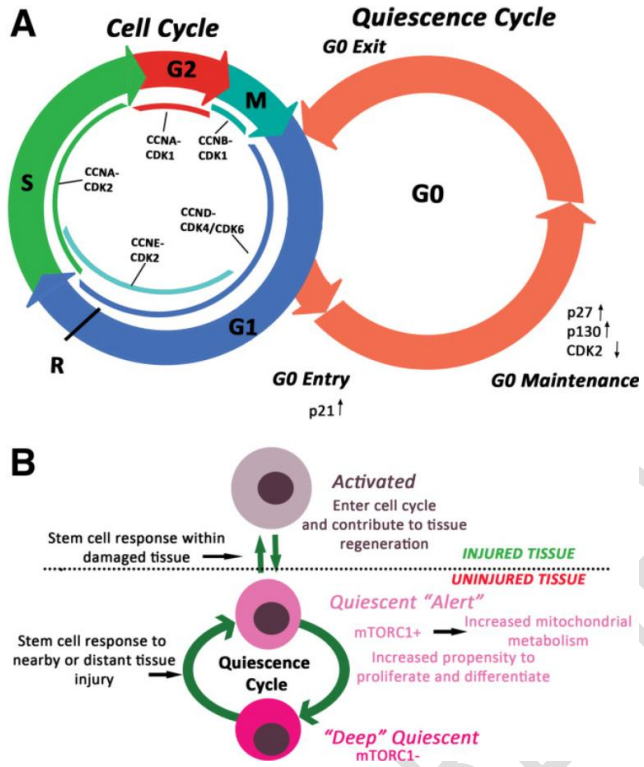

Figure 1. The cell cycle and the quiescence cycle [13]

Targeting Cancer Stem Cell Pathways for Cancer Therapy

Wnt signaling pathway in CSCs

Wnt includes large protein ligands that affect diverse processes, such as the establishment of cell polarity and cell fate [14]. The Wnt pathway is highly complex and evolutionarily conserved and includes 19 Wnt ligands and more than 15 receptors [15]. The Wnt signaling pathway can be divided into canonical Wnt signaling (via the FZD-LRP5/6 receptor complex, leading to derepression of β-catenin) and noncanonical Wnt signaling (via FZD receptors and/or ROR1/ROR2/RYK co-receptors, activating PCP signaling, RTK, or Ca2+ cascades) [16]. In canonical Wntsignaling , in the absence of Wnt ligands (**Figure 2.** inactive Wnt signaling state), β-catenin is phosphorylated by glycogen synthase kinase 3β (GSK3β), which causes degradation of β-catenin via ubiquitination of β-TrCP200 and inhibits translocation of β-catenin from the cytoplasm to the nucleus [17]. In contrast, in the presence of Wnt ligands (e.g., Wnt3a and Wnt1), they associate with the Fzd receptor and LRP coreceptor (**Figure 3.** active Wnt signal).

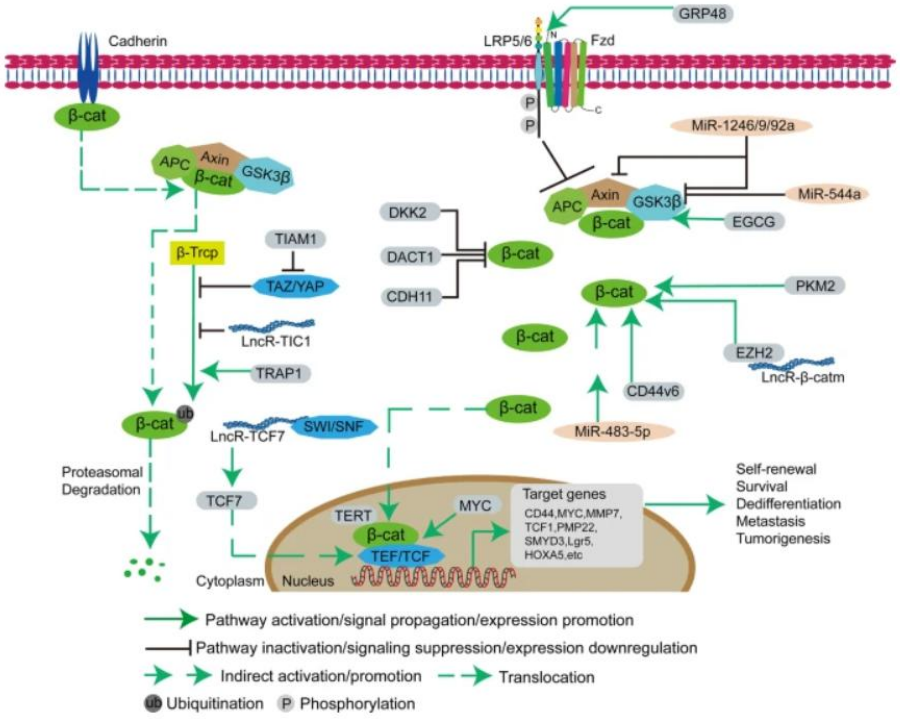

Figure 2. Inactive Wnt signaling state

The LRP receptor is phosphorylated by GSK3β and CK1α [18]. β-Catenin is released from the Axin complex to enter the nucleus. Additionally, β-catenin associates with LEF/TCF and increases the recruitment of histone modifying coactivators, such as BCL9, Pygo, CBP/p300, and BRG1, to activate transcription. Noncanonical Wnt signaling does not involve β-catenin. During Wnt/PCP signaling, Dvl is activated through binding of Wnt ligands and ROR-Frizzled receptors [19]. Dvl inhibits binding of the small GTPase Rho and the cytoplasmic protein DAAM1 [20]. The small GTPases Rac1 and Rho together trigger ROCK (Rho kinase) and JNK (c-Jun N-terminal kinase). This results in cytoskeletal rearrangements and/or transcriptional responses [21]. Wnt/Ca2+ signaling is activated by G protein-stimulated phospholipase C activity, which results in intracellular calcium fluxes and downstream calcium-dependent cytoskeletal and/or transcriptional responses [22, 23].

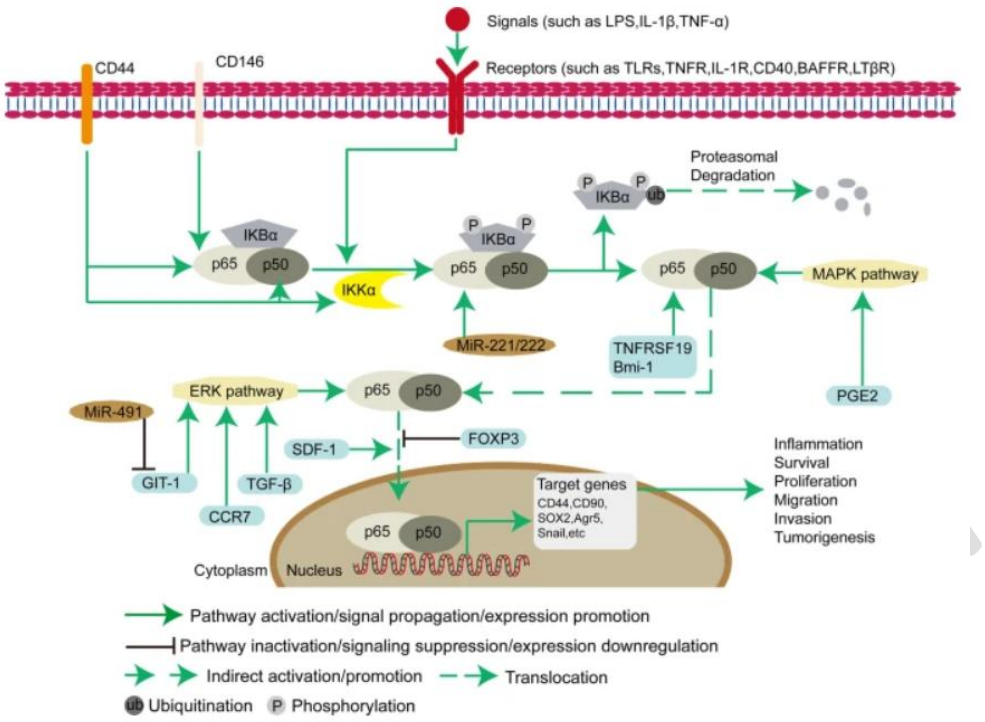

Figure 3. Active Wnt signaling state

NF-κB signaling pathway in CSCs

The NF-κB pathway has important relationships regulating inflammation, self-renewal, or maintenance and metastasis of CSCs (**Figure 4**). CD44+ cells promote self-renewal, metastasis, and maintenance of CSCs by increasing the expression of RelA, RelB, and IKKα and mediating nuclear activation of the p50/RelA dimer (p50/p65) [24]. High levels of NIK can induce activation of the noncanonical cell NF-κB pathway to regulate self-renewal and metastasis of breast CSCs [25]. In addition, stromal cell-derived factor-1 (SDF-1) also has a similar effect by regulating the translocation of p65 from the cytoplasm to the nucleus [26]. The inflammatory mediator prostaglandin E2 (PGE2) contributes to the formation, maintenance, and tumor metastasis by activating NF-κB via the EP4-PI3K (phosphoinositide 3-kinase) and EP4-MAPK pathways in colorectal CSCs [27]. Chemokines, proinflammatory low molecular weight cytokines, are important mediators of cell proliferation, metastasis, and apoptosis [28]. Receptors C-C chemokine 7 interacts with chemokine ligand 21 to inhibit apoptosis and induce survival and migration in CD133+ pancreatic cancer-like cells by increasing the expression of extracellular signal-regulated kinase 1/2 (Erk1/2) and p65.295

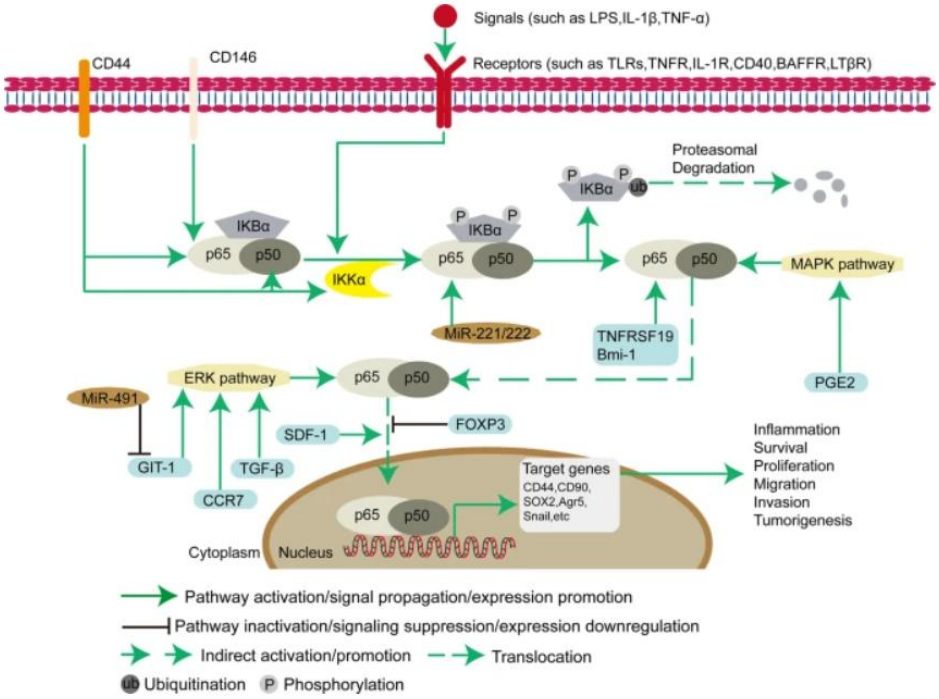

Figure 4. NF-κB signaling pathway in CSCs

In addition, viral integration sites Moloney murine leukemia specific B cells 1 (Bmi-1) also increases p65 protein in gastric CSCs.296 MicroRNAs also play an important role in promoting CSC proliferation. Mir-221/222 promotes self-renewal, migration, and invasion in breast CSCs by inhibiting PTEN expression and subsequently inducing AKT phosphorylation, resulting in increased p65, p-p65, and COX2 [29].

JAK-STAT signaling pathway in CSCs

The JAK/STAT pathway is evolutionarily conserved. This pathway promotes ESC survival, self-renewal, hematopoiesis, and neurogenesis [30]. This pathway is also activated in CSC. Sustained STAT3 activation significantly increases cell survival and stemness maintenance in breast CSCs [31]. IL-10 induces cell self-renewal, migration, and invasion in non-small cell lung CSCs [32]. IL-6 activates JAK1/STAT3 pathway in high CD126+ endometrial ALDH CSCs [33]. Additionally, IL-6 also induces the conversion of non-stem cancer cells into cancer stem-like cells in breast cancer by activating the downstream gene Oct4 [34]. Oct4 also activates the JAK1/STAT6 pathway in ovarian CSCs [35]. In CD44+CD24− and colorectal breast CSCs, erythropoietin, and IL-6 activate the

JAK2/STAT3 pathway [36, 37, 38]. Retinol-binding protein 4 activates JAK2/STAT3 signaling via the STRA6 receptor in colon CSCs [39]. HIF-1α promotes self-renewal of glioma stem-like cells via the JAK1/STAT3 pathway [40]. AJUBA is a scaffolding protein that participates in the regulation of cell adhesion, differentiation, proliferation, and migration and promotes cell survival and proliferation of colorectal CSCs via the JAK1/STAT1 pathway [41].

PI3K/AKT/mTOR signaling pathway in CSCs

Phosphatidylinositol-3-kinase (PI3K) is an intracellular phosphatidylinositol kinase [42]. It consists of the p85 regulatory subunit and the p110 catalytic subunit, which have serine/threonine (Ser/Thr) kinase and phosphatidylinositol kinase activities [43]. AKT is a serine/threonine kinase consisting of of three isoforms: AKT1, AKT2, and AKT3 [44]. The AKT protein is an important effector of PI3K and is activated directly in response to PI3K. One of the major downstream target genes of AKT is the mammalian target of rapamycin (mTOR) complex, which is a conserved serine/threonine kinase. It forms two distinct multiprotein complexes: mTORC1 and mTORC2 [45]. mTORC1 consists of mTOR, raptor, mLST8, and two negative regulators, PRAS40 and DEPTOR [46, 47]. mTORC2 phosphorylates AKT at serine residue 473, leading to full activation of the AKT protein [48].

Research shows that mutations in the PTEN gene cause inhibition of PI3K/mTOR signaling in glioblastoma multiforme. However, deletion of PTEN in neural stem cells can lead to a neoplastic phenotype that includes increased cell growth, resistance to cell apoptosis, and increased migratory and invasive properties in vivo [49]. Inactivation of PTEN and activation of protein kinase B have been found in other solid tumors, such as myeloproliferative neoplasia and leukemia [50]. Therefore, the PI3K/mTOR signaling pathway is essential for cell proliferation and survival. In several types of cancer, such as non-small cell lung cancer [51], breast cancer [52], prostate cancer [53], Burkitt's lymphoma [54], esophageal adenocarcinoma [55], and colorectal cancer [56] abnormal activation of PI3K/mTOR signaling was found.

Targeting Unique FeaturesQCCs to Overcome Resistance and Recurrence in Cancer Therapy

The optimal result of cancer treatment is the eradication of all cancer cells, both proliferating cancer cells and quiescent cancer cells. Because QCC have different characteristics compared with proliferating cells, efforts to develop QCC eradication strategies are highly recommended. We collected several characteristics; however, we cannot cover them all, as they have all been studied separately in different studies.

(a) Quiescent cancer cells exhibit altered mitochondrial activity

There are several studies that have shown that inhibition of mitochondrial OXPHOS is a promising strategy to combat quiescent cancer cells in hypoxic and nutrient-deficient environments. In one study, experiments developed a melanoma cell model in which the endogenous cell cycle inactive marker p27 gave increased GFP signals and the endogenous cell cycle active marker ki-67 gave increased mCherry signals. Using this cell model, the authors identified a group of cancer cells that express low levels of ki-67 and high levels of p27, which are thought to be in a quiescent state. Compared with other cells, these QCC showed high levels of c-Myc expression and stimulated mitochondrial OXPHOS activity by transactivating genes encoding OXPHOS enzymes, including isocitric dehydrogenase subunit 3 (IDH3) [57].

Further inhibition of mitochondrial OXPHOS by a small molecule inhibitor of mitochondrial complex I, IACS-010759 [57], can lead to reduced cell viability in quiescent cells, whereas they do not significantly affect the viability of cells active in the cell cycle [58], indicating that targeting OXPHOS mitochondria can overcome drug resistance in QCC. Similar findings have also been reported in other studies. For example, using a glucose-deficient multicellular tumor spheroid (MCTS) model with a QCC population at the core, Senkowski et al. screened 1,600 compounds with a documented clinical history and identified five molecules showed selective MCTS activity: nitazoxanide, niclosamide, closantel, pyrvinium pamoate, and salinomycin.Further experiments revealed that all five identified compounds inhibited mitochondrial respiration, indicating that the MCTS containing QCC population relies on oxidative phosphorylation rather than just

glycolysis [59]. In another study, the authors created three different models including monolayer, proliferative MCTS, and silent MCTS using HCT116 colon carcinoma cancer cells and profiled gene expression on a panel of compounds targeting various processes (mitochondrial inhibitors, autophagy inhibitors, kinase inhibitors, mTOR inhibitors , MEK inhibitors, etc.). The authors further found that after exposure to OXPHOS inhibitors, the mevalonate pathway was significantly upregulated. The combination of the cholesterol synthesis inhibitor zaragozic acid with the mitochondrial inhibitor nitazoxanide resulted in a strong reduction in colony formation. However, the combination of nitazoxanide with irinotecan, the PI3K/mTOR dual inhibitor BEZ235, or the autophagy inhibitor Lys05 did not cause increased toxicity against quiescent MCTS, indicating that inhibition of the mevalonate pathway is a promising strategy to potentiate the effects of OXPHOS. inhibitors against QCCs [60].

Other studies have also shown that disruption of mitochondrial fatty acid β-oxidation (FAO) can induce apoptosis in quiescence-induced cells and inhibit the return to proliferation, suggesting that targeting mitochondrial metabolism in QCC may reveal basic principles in cell plasticity and potential new outcomes. therapeutic options [61]. We also reported that VLX600, a mitochondrial inhibitor, was able to eliminate not only proliferating cancer cells but also quiescent cancer cells, due to the induction of a bioenergetic catastrophe following mitochondrial inhibition [62]. Mitochondria are the main source of ATP production and also play an important role in building macromolecules, regulating signaling processes, maintaining ROS homeostasis, regulating intrinsic cell apoptosis, and cancer metastasis [63, 64]. Therefore, it is reasonable to suggest that mitochondria are indispensable in QCC, and targeting mitochondria, such as OXPHOS, may be a promising strategy to eliminate QCC [57,58,59,60,65].

(b) Quiescent cancer cells cannot tolerate aggravated autophagy

In solid tumors, quiescent cancer cells arelocated in areas away from blood vessels that lack nutrients and oxygen. Previous studies showed that VLX600 exhibited extreme toxicity against quiescent cancer cells due to mitochondrial inhibition and autophagy induction [62]. In another study, quiescent cancer cells treated with inhibitors of ULK1, a key kinase that activates autophagy, in

combination with standard chemotherapy treatment (CPT-11), underwent apoptosis and were unable to regrow after treatment [66].

Another example is saikosaponin A, a Bupleurum-derived compound capable of exacerbating autophagy by inactivating Akt-mTOR signaling and effectively eliminating multidrug-resistant quiescent prostate cancer cells. Moreover, administration of saikosaponin A during the docetaxel treatment interval resulted in robust cell death in vitro and in vivo, suggesting that saikosaponin A may improve therapeutic efficacy and prevent cancer recurrence by targeting QCCs [67].

(c) Quiescent cancer cells show high levels of DYRK1B

The dual-specificity tyrosine-regulated kinase (DYRK) family, consisting of DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4, belongs to the CMGC group that includes cyclin-dependent kinases (CDKs), mitogen-activated protein kinase (MAPK), glycogen synthase kinases (GSK), and CDK-like kinases (CLKs) [68]. DYRK1B family members have shown a strong increase when tumor cells exit the cell cycle after mitogen deprivation or pharmacological inhibition of proliferation pathways in various types of cancer cells such as breast, colon carcinoma, melanoma, pancreatic, and ovarian cancer cells[69,70,71]. In contrast, decreasing DYRK1B levels by RNA interference allows C2C12 myoblasts to re-enter the cell cycle [72], indicating that DYRK1B plays an important role in maintaining cancer cells in a quiescent state. The underlying mechanisms may be complex, but some evidence suggests that DYRK1B is able to control the S phase checkpoint by stabilizing the cyclin-dependent kinase inhibitor p27Kip1 and inducing cyclin D degradation [73, 74]. DYRK1B also stabilizes the DREAM complex (DP, RB, E2F, and MuvB), and is an important coordinator in maintaining the cell's quiescent G0 state, by phosphorylating LIN52 at Ser28 [75].

In addition, DYRK1B has pro-survival functions by upregulating antioxidant gene expression and reducing intracellular reactive oxygen species levels [76, 77]. Substantial evidence suggests that depletion or inhibition of DYRK1B promotes cell cycle re-entry and increases apoptosis of quiescent cancer cells with high DYRK1B expression [78, 79, 80]. Furthermore, DYRK1B inhibitors were shown to sensitize cells to the cytotoxic effects of anticancer drugs targeting proliferating cells [81,82]. In conclusion, targeting increased DYRK1B levels in QCC may

disrupt the quiescent state and eliminate it further through anticancer reagents targeting proliferating cells.

(d) Quiescent cancer cells show the upregulation of the c-YES/YAP signaling axis

c-YES is a cytoplasmic non-receptor protein belonging to the SRC kinase (SFK) family that has been shown to have oncogenic properties and serve as a biomarker in various types of tumors [83]. c-YES is overexpressed in cancer cells and is associated with poor prognosis [84,85]. Amplification of c-YES also occurs in some patients treated with EGFR and ALK inhibitors who become resistant to targeted therapy [86, 87]. Recent studies show that, in the HT29 colon cancer cell line, a population of 5FU-resistant clonal cells can enter a reversible quiescent G0 state when re-exposed to higher concentrations of 5FU. These quiescent cells showed upregulated expression levels of c-YES/YAP signaling. In addition, clinical results showed that YES1 and YAP transcript levels were higher in colon cancer patients with liver metastases after 5FU-based neoadjuvant chemotherapy, which also had a positive correlation with colon cancer recurrence and shorter patient survival [84].

Further studies showed that 5FU-induced quiescent cancer cells expressed high levels of YAP and reduced levels of cyclin E1 and c-Myc, which were associated with shorter disease-free times and overall survival [88]. Overall, the c-Yes/YAP signaling pathway can be considered as a potential therapeutic target to kill drug-resistant quiescent cancer cells.

(e) Quiescent cancer cells show immune evasion capacity

Metastasis usually occurs after resection of the primary tumor, with a small number of cancer cells spreading and persisting as a latent entity through unknown mechanisms. Researchers isolated latent carcinoma cells (LCC) from ancient human lung and breast tumors and found that these LCC cells readily entered a quiescent state in low-mitogen medium (MLM, 2% serum), whereas markers of apoptosis (e.g., caspase - 3) remains unchanged for months as a potential entity in the associated organ. These LCC quiescent cells still retain tumorigenic and metastasis-inducing potential. Further studies revealed that these

QCC express DKK1 protein, which further leads to downregulation of NK cell activators and escape from immune surveillance [89].

These findings suggest that selectively reactivating NK cell ligands in quiescent metastatic cells can trigger immunological elimination of latent metastases. Except for the ability to escape immune surveillance, recent studies also revealed that QCC, which expresses high levels of the silent marker p27 in breast tumor cells, is able to resist T cell attacks by establishing an immunosuppressive niche. These QCCs showed high levels of genes associated with chemoresistance (Car9, Kdm5a, and Kdm5b), hypoxia (Hif1a), and glycolysis (glucose transporter Slc2a1 or Glut1). However, the expression levels of Cd81, Il12a, and Il12b, which represent key cytokines for T cell responses [90], were lower in the QCC niche, indicating that QCC may induce immunotherapy resistance by regulating a local hypoxic immunosuppressive environment to block T cell function, and restore T cell function promises to eliminate QCC and thereby counter immunotherapy resistance [91].

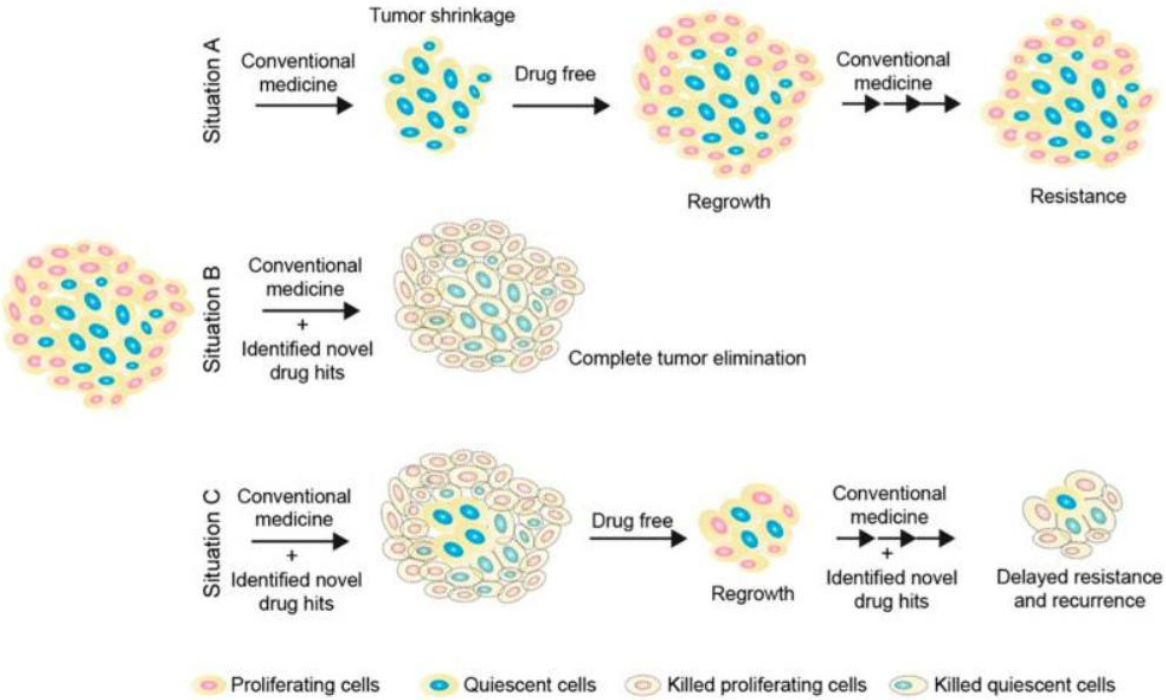

Figure 5. Elimination of tumor cells by targeting both proliferative and quiescent cancer cell populations.

Conclusion

Quiescent cancer cells can evade most cancer treatments and have been associated with recurrence and resistance to stem cell cancer because they can re-enter a proliferative state when conditions are favorable. This accumulation of research aimed at finding therapeutic options for QCC in solid tumors has revealed several clues to overcome resistance and recurrence in cancer therapy. Here, we review and discuss recent research advances in the treatment of QCC including reactivating quiescent cancer cells and eliminating them through cell cycle-dependent anticancer reagents, modulating the quiescence-to-proliferation switch; and eliminate QCC by targeting its unique features.

Considering the many obstacles faced by QCC to the treatment of solid tumors, there is a great need to understand the characteristics of QCC, decipher the mechanisms that regulate the proliferative-quiescent transition in cancer cells, and develop new strategies to eliminate remaining QCC in solid cancer cells. tumor. This remains a long-term challenge in the future.

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