Targeting Unique Features of Quiescent Cancer Stem Cells to Overcome Resistance and Recurrence in Cancer Therapy: A Review

Abstract

Quiescent cancer stem cells (QCSC) are non-proliferating cells that survive in the G0 phase and have low ki-67 expression and high p27 expression. QCSCs have the ability to evade most chemotherapy, and some subsequent treatments may result in a higher proportion of quiescent cancer stem cells in the tumor. QCSCs are also associated with cancer recurrence rates because they can re-enter the cell cycle to proliferate when tumor environmental conditions are favorable. QCSCs cause high rates of drug resistance and tumor recurrence, therefore it is necessary to understand the properties of QCSCs. QCSCs have a mechanism that regulates the transition between the proliferative phase and the stationary phase in cancer cells, therefore it is necessary to find new treatments to eliminate QCSCs in tumors. In this review, the authors discuss the mechanisms of QCSCs in inducing drug resistance and tumor recurrence as well as therapies to target QCSCs so that the rate of drug resistance and tumor recurrence can be reduced, including in this review: (i) identifying reactive quiescent cancer cells and eliminating them through anticancer reagents. cell cycle dependent; (ii) modulating the transition from the quiescent to the proliferative phase; and (iii) eliminate QCSC by targeting its unique features. Targeting cancer cells that are in proliferating and stationary phase may ultimately be used as a more effective therapeutic strategy for cancer treatment.

Keywords: quiescent cancer stem cells, targeting unique features, targeted therapy

Introduction

“Cancer is a chronic disease that is very threatening to human life. Many strategies have been discovered in cancer treatmentincluding chemotherapy, radiotherapy, surgery and targeted therapy. The incidence of cancer in women has stabilized and decreased slightly in men in the last decades, and cancer death rates have also decreased” [1]. However, not all types of cancer can be treated with traditional cancer treatment [2]. Recurrence, metastasis, resistance, heterogeneity to chemotherapy, evasion of immunological surveillanceand radiotherapyare the main factors causing cancer treatment failure [3]. “This failure can be described by the characteristics of the cancer stem cells” [4]. “Cancer stem cells can cause cancer recurrence, metastasis,radiation resistance, and multidrug resistancethrough their ability to survive in the G0 phase so that in a favorable environment they can give rise to new cancer” (5). Therefore, cancer stem cells are currently considered the most important target for cancer treatment.

“Cancer stem cells were first identified in leukemia patients in the 1990s,andisolated through the expression of surface markers CD34+ and CD38−“ [6,7]. “Cancer stem cells can express surface markers such as nestin, CD44, and CD133 and are found in many non-solid and solid tumors” [8,9]. “CSCs can proliferate to produce tumors through self-renewal mechanisms and differentiation into several cellular subtypes” [10].Intracellular and extracellular factors are factors that can be used as drug targets for cancer treatment because they can control CSC activity [11]. To understand the properties of CSCs, through this review we summarize the therapeutic methods targeting cancer stem cells that are effective for cancer therapy in both basic biomolecular research and clinical studies.

Characteristics of Quiescent Cancer Stem Cells

“Quiescent cancer stem cells (QCSC) are non-proliferating cells arrested in the G0 phase, characterized by low ki-67 expression and high p27 expression. QCSC has the ability to avoid most chemotherapy, and some subsequent treatments may result in a higher proportion of quiescent cancer stem cells in the tumor. QCSC is also related to cancer recurrence rates because they can re-enter the cell cycle to proliferate when tumor environmental conditions are favorable. QCSCs are also associated with cancer recurrence rates because they can re-enter the cell cycle to proliferate when tumor environmental conditions are favorable. QCSCs cause

high rates of drug resistance and tumor recurrence, therefore it is necessary to understand the properties of QCSCs". [92]"QCSCs have a mechanism that regulates the transition between the proliferative phase and the stationary phase in cancer cells, therefore it is necessary to find new treatments to eliminate QCSCs in tumors. In this review, the authors discuss the mechanisms of QCSCs in inducing drug resistance and tumor recurrence as well as therapies to target QCSCs so that the rate of drug resistance and tumor recurrence can be reduced, including in this review: (i) identify reactive quiescent cancer cells and eliminate them using cell cycle-dependent anticancer reagents; (ii) modulating the transition from the quiescent to the proliferative phase; and (iii) eliminate QCSC by targeting its unique features. Targeting cancer cells that are in proliferating and stationary phase may ultimately be used as a more effective therapeutic strategy for cancer treatment" [12].

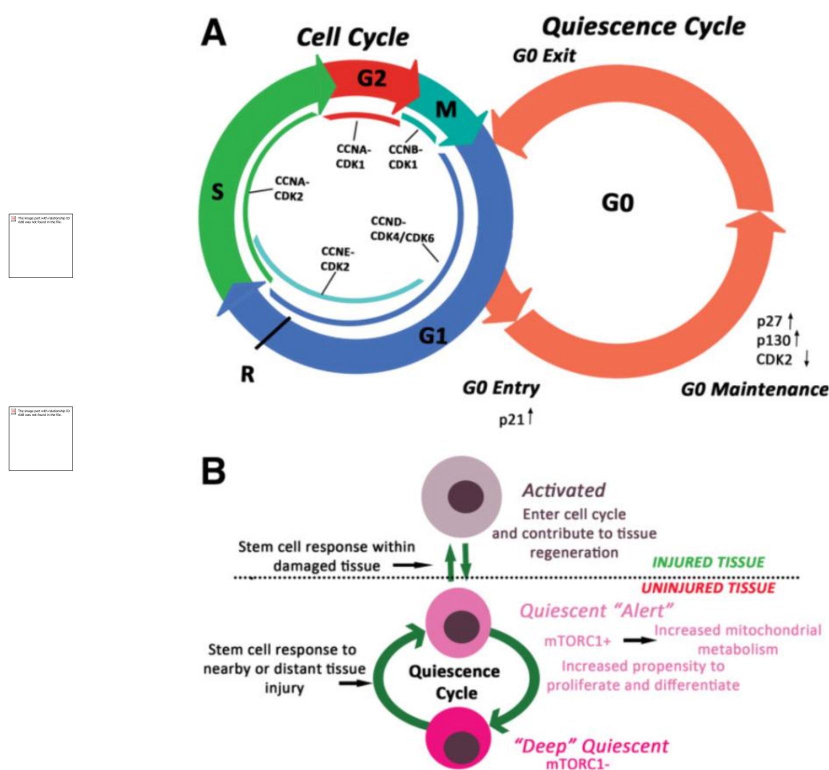

Figure 1. The cell cycle and the quiescence cycle [13]

Cancer Stem Cell Pathways as targets for Cancer Therapy

Wnt signaling pathway in CSCs

"Wnts include large protein ligands that influence the establishment of cell polarity and cell fate" [14]. "The Wnt pathway is very complex consisting of 19 Wnt ligands and more than 15 receptors" [15]. "The Wnt signaling pathway consists of canonical Wnt signaling (via the FZD-LRP5/6 receptor complex, leading to β-catenin derepression) and non-canonical Wnt signaling (via the FZD receptor and/or ROR1/ROR2/RYK co-receptors, activating PCP signaling , RTK, or Ca2+ cascade)" [16]. In canonical Wnt signaling, i.e. in the absence of Wnt ligands (**Figure 2**. Inactive Wnt signaling state), glycogen synthase kinase 3β (GSK3β) phosphorylates β-catenin and through ubiquitination of β-TrCP200 results in degradation of β-catenin as well as inhibiting β-catenin translocation from the cytoplasm to the nucleus [17]. In contrast, in the presence of Wnt ligands such as Wnt3a and Wnt1, they associate with the Fzd receptor and LRP co-receptor (**Figure 3**. Active Wnt signaling).

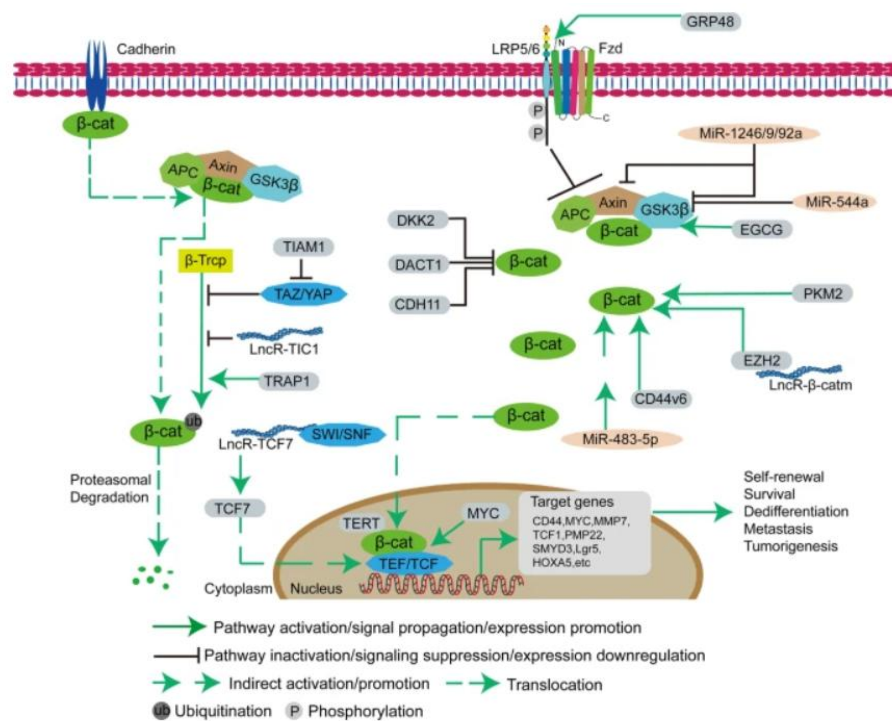

Figure 2. Inactive Wnt signaling state [17]

GSK3β and CK1α are phosphorylated by LRP receptors [18]. "The Axin complex releases β-Catenin to enter the nucleus. In addition, the association of β-catenin with LEF/TCF results in increased recruitment of histone modifying coactivators, such as Pygo, BCL9, CBP/p300, and BRG1, to carry out transcriptional activation.

β-catenin is not involved in noncanonical Wnt signaling. During Wnt/PCP signaling, Dvl activation occurs through the binding mechanism of Wnt ligands and ROR-Frizzled receptors” [19]. Dvl has a role in inhibiting the binding of the cytoplasmic protein DAAM1 and the small GTPase Rho [20], where the small GTPase Rac1 and Rho can trigger JNK (c-Jun N-terminal kinase) and ROCK (Rho kinase). This leads to rearrangement of transcriptional and/or cytoskeletal responses [21]. “Phospholipase C activity activates G protein-induced Wnt/Ca2+ signaling. This leads to downstream calcium-dependent cytoskeletal and/or transcriptional responses as well as calcium fluxes within the cell” [22, 23].

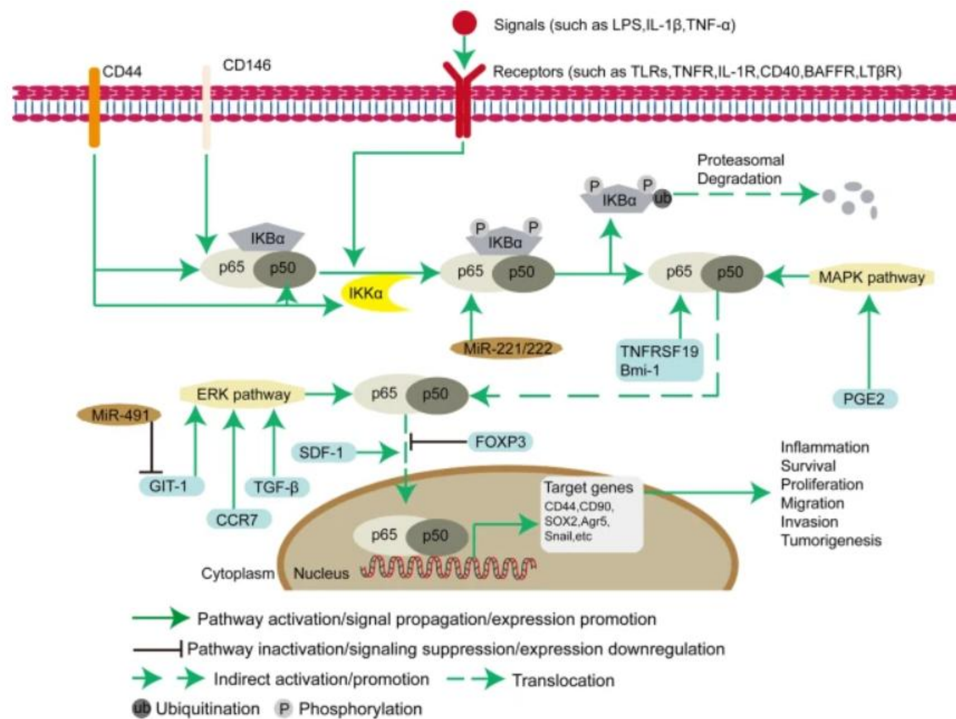

Figure 3. Active Wnt signaling state [17]

NF-κB signaling pathway in CSCs

The NF-κB pathway has an important role in regulating CSC self-renewal, inflammation, maintenance and metastasis (**Figure 4**). CD44+ cells induce self-renewal, CSC maintenance and metastasis, through increasing the expression of IKKα, RelA, RelB, as well as mediating nuclear activation of the p50/RelA dimer (p65/p50) [24]. “Activation of the NF-κB pathway of noncanonical cells is induced by high levels of NIK to regulate self-renewal and metastasis of breast CSCs” [25].

“Stromal cell-derived factor-1 (SDF-1) also has a role in regulating p65 translocation from the cytoplasm to the nucleus” [26]. “In colorectal CSCs, Prostaglandin E2 (PGE2) contributes to tumor formation, maintenance, and metastasis through activating NF-κB in the EP4-PI3K (phosphoinositide 3-kinase) and EP4-MAPK pathways” [27]. “In cell proliferation, metastasis, and apoptosis involving important mediators such as chemokines, low molecular weight proinflammatory cytokines” [28]. “Interaction between C-C chemokine receptor 7 with chemokine ligand 21 results in inhibition of apoptotic mechanisms and induces survival and migration in CD133+ pancreatic cancer-like cells by increasing the expression of p65 and extracellular signal-regulated kinase 1/2 (Erk1/2)” [28].

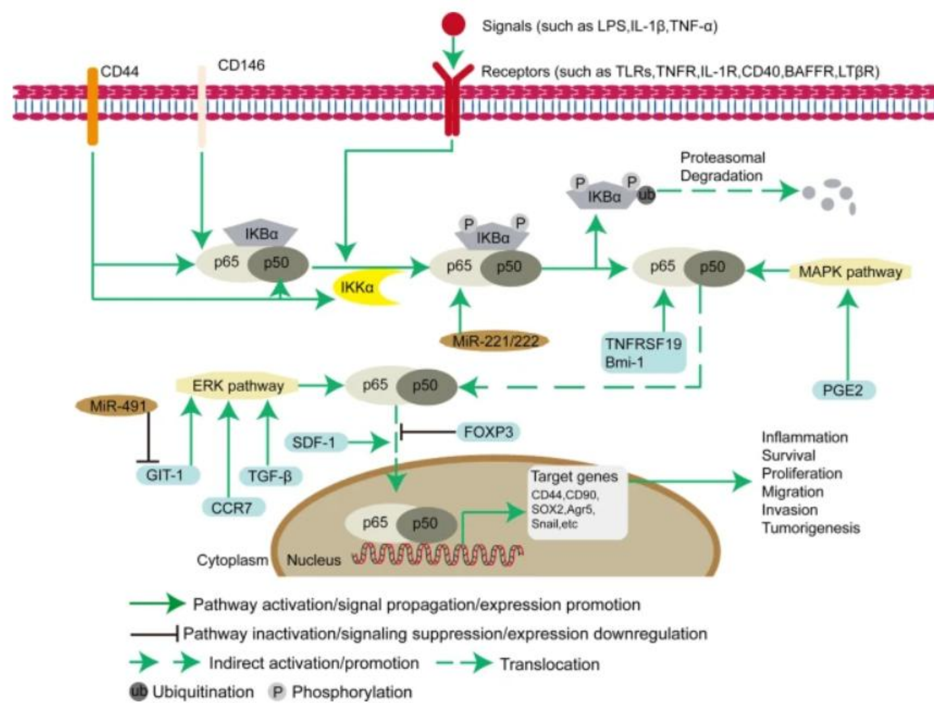

Figure 4. NF-κB signaling pathway in CSCs [24]

In addition, in gastric CSCs there was an increase in p65 protein in the integration site of the specific B cell murine leukemia virus Moloney 1 (Bmi-1) as well as MicroRNA also plays an important role in promoting CSC proliferation [28]. Inhibition of PTEN expression and subsequent induction of AKT phosphorylation resulting in increased p65, p-p65, and COX2 resulted in Mir-221/222 promoting self-renewal, migration, and invasion in breast CSCs [29].

JAK-STAT signaling pathway in CSCs

The JAK/STAT pathway induces ESC survival, self-renewal, hematopoiesis as well as neurogenesis [30]. In CSCs, this pathway is activated through sustained activation of STAT3, and significantly has the effect of enhancing cell survival and stemness maintenance in breast CSCs [31]. Cell renewal, migration, and invasion in non-small cell lung CSCs are induced by IL-10 [32]. The JAK1/STAT3 pathway in CD126+ high ALDH endometrial CSCs is activated by IL-6 [33]. In addition, IL-6 through activation of the downstream gene Oct4 in breast cancer induces the conversion of non-stem cancer cells into cancer stem-like cells [34]. The JAK1/STAT6 pathway in ovarian CSCs is also activated by Oct4 [35]. Erythropoietin and IL-6 activate the JAK2/STAT3 pathway in CD44+CD24− as well as breast and colorectal CSCs [36, 37, 38]. JAK2/STAT3 signaling is activated by retinol-binding protein 4 via the STRA6 receptor in colon CSCs [39]. Through the JAK1/STAT3 pathway, HIF-1α promotes self-renewal of glioma stem-like cells [40]. AJUBA is a scaffolding protein that plays a role in the regulation of cell adhesion, proliferation, differentiation, and migration and through the JAK1/STAT1 pathway plays a role in increasing cell survival and proliferation of colorectal CSCs [41].

PI3K/AKT/mTOR signaling pathway in CSCs

Phosphatidylinositol-3-kinase (PI3K) is an intracellular phosphatidylinositol kinase [42]. Phosphatidylinositol-3-kinase (PI3K) consists of the p110 catalytic and p85 regulatory subunits, which have serine/threonine (Ser/Thr) kinase and phosphatidylinositol kinase activities [43]. AKT is a serine/threonine kinase that has three isoforms including AKT1, AKT2, and AKT3 [44]. The AKT protein is an important effector for PI3K and can also be a response to PI3K. The mammalian target of rapamycin complex (mTOR) is the main downstream target gene of AKT, which is a conserved serine/threonine kinase. The mammalian target of rapamycin complex (mTOR) forms two different multiprotein complexes including mTORC1 and mTORC2 [45]. mTORC1 consists of mTOR, raptor, mLST8, and two negative regulators including DEPTOR and PRAS40 [46, 47]. At serine residue 473, mTORC2 phosphorylates AKT leading to full activation of the AKT protein [48].

Recent research shows that in glioblastoma multiforme, mutations in the PTEN gene have the effect of inhibiting PI3K/mTOR signaling. However, deletion of the PTEN gene in neural stem cells can cause increased cell growth, invasiveness in vivo, resistance to cell apoptosis as well as increased cell migration properties [49]. Inactivation of PTEN and activation of protein kinase B have been found in myeloproliferative neoplasia and leukemia [50]. Therefore, the PI3K/mTOR signaling pathway is critical in regulating cell proliferation and survival. In non-small cell lung cancer [51], breast cancer [52], prostate cancer [53], Burkitt lymphoma [54], esophageal adenocarcinoma [55], and colorectal cancer [56] improper activation of PI3K/mTOR signaling normal was found.

Targeting Unique FeaturesQCCs to Overcome Resistance and Recurrence in Cancer Therapy

Eradicating all cancer cells, both proliferating cancer cells and quiescent cancer cells, is the main goal of optimal cancer treatment (**Figure 5)**. Because QCC and proliferating cells have different characteristics, it is necessary to find a QCC elimination strategy. We found several characteristics of QCC, but cannot cover all of them, because they have been studied separately in different studies.

(a) Quiescent cancer cells exhibit altered mitochondrial activity

One study showed that inhibiting mitochondrial OXPHOS is a effective strategy to against cancer cells that reside in environments of nutrient deprivation and hypoxia. A study showed that in melanoma cells, the endogenous cell cycle inactive marker p27 induced an increase in GFP signal and the endogenous cell cycle active marker ki-67 caused an increase in mCherry signal.The authors identified a group of cancer cells expressing 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 show high levels of c-Myc expression and stimulate mitochondrial OXPHOS activity by transactivating genes encoding OXPHOS enzymes, including isocitric dehydrogenase subunit 3 (IDH3) [57].

Inhibition of mitochondrial OXPHOS has the effect of reducing cell viability in quiescent cells by a small molecule inhibitor of mitochondrial complex I, IACS-010759 [57], whereas it does not significantly affect the viability of cells active in

the cell cycle [58]. This suggests that overcoming drug resistance in QCC can be done by targeting mitochondrial OXPHOS. Similar results have been shown by other studies, where glucose-deficient multicellular tumor spheroids (MCTS) with the QCC population as the core, then screened 1,600 compounds with a documented clinical history and identified five molecules that showed selective MCTS activity including niclosamide, nitazoxanide, closantel, pyrvinium pamoate, and salinomycin.These experiments showed that the five identified compounds were proven to inhibit mitochondrial respiration. In this study it can also be concluded that MCTS containing the QCC population is very dependent on oxidative phosphorylation from glycolysis [59]. In another study, three different models, namely monolayer, proliferative MCTS, and quiescent MCTS, used HCT116 colon carcinoma cancer cells and examined the gene expression profiles on a panel of compounds targeting various processes (mitochondrial inhibitors, kinase inhibitors, autophagy inhibitors, mTOR inhibitors, MEK, etc.). Further research found that after exposure to OXPHOS inhibitors, the mevalonate pathway was significantly upregulated. The combination of the mitochondrial inhibitor nitazoxanide and the cholesterol synthesis inhibitor zaragozic acid can result in a strong reduction in cancer cell 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, but suggests that inhibition of the mevalonate pathway is a promising strategy to optimize the effects of OXPHOS inhibitors on QCC [60].

Other studies also show that disruption of mitochondrial fatty acid β-oxidation (FAO) can induce apoptosis in cells. Targeting mitochondrial metabolism in QCC could be a fundamental principle of cell plasticity and a potential novel therapeutic option [61]. We also reported that the mitochondrial inhibitor VLX600, was able to eliminate not only proliferating cancer cells but also quiescent cancer cells, due to the bioenergetic disruption effect following mitochondrial inhibition [62]. The main source of ATP production inmitochondria. Mitochondria also play an important rolein building macromolecules, regulating signaling processes, regulating intrinsic cell apoptosis, maintaining ROS homeostasis and cancer metastasis [63, 64]. Therefore, it makes sense that the presence of mitochondria is necessary in QCC.

Mitochondrial targeting, such as OXPHOS, may be a promising strategy to eliminate QCC in cancer cells [57,58,59,60,65].

(b) Quiescent cancer cells cannot tolerate exacerbated autophagy

Quiescent cancer cells in solid tumors are usually located in areas far from blood vessels, causing a state of lack of nutrients and oxygen. Previous studies showed that VLX600 exhibited high toxicity effects against quiescent cancer cells through inhibiting mitochondrial induction mechanisms and autophagy [62]. In another study, quiescent cancer cells were treated with an inhibitor of ULK1, a key kinase that activates autophagy in combination with standard chemotherapy treatment (CPT-11), and were able to undergo apoptosis and were unable to regrow after treatment [66].

Saikosaponin A is a Bupleurum derivative compound that deactivates the Akt-mTOR signal and can worsen autophagy and can effectively eliminate silent prostate cancer cells that are resistant to various drugs. In addition, administration of saikosaponin A can be administered during the docetaxel treatment interval and can cause potent cell death in vitro and in vivo. This research shows that saikosaponin A can increase the effectiveness of therapy and prevent cancer recurrence by targeting QCCs [67].

(c) Quiescent cancer cells have high levels of DYRK1B

A family of tyrosine-regulated kinases that have dual specificity belongs to the CMGC(DYRK) group consisting of DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4 which includes mitogen-activated protein kinase (MAPK), cyclin-dependent kinases (CDKs), glycogen synthase kinase (GSK), and CDK-like kinases (CLKs) [68]. Studies have shown that there is a strong increase in DYRK1B family members 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, melanoma, colon, pancreatic and ovarian carcinomas [69, 70, 71]. Conversely, due to RNA interference there is a decrease in DYRK1B levels which allows C2C12 myoblasts to re-enter the cell cycle [72]. DYRK1B plays an important role in maintaining cancer cells in the stationary

phase. DYRK1B is able to control the checkpoint in S phase by stabilizing the cyclin-dependent kinase inhibitor p27Kip1 and inducing cyclin D degradation [73, 74]. DYRK1B also stabilizes the DREAM complex consisting of E2F, DP, RB, and MuvB, which is an important coordinator in phosphorylating LIN52 at Ser28 to keep the cell in the G0 quiescent state [75].

In addition,upregulating antioxidant gene expression and reducing intracellular reactive oxygen species levels, DYRK1B has a pro-survival role [76,77]. Substantial evidence suggests that quiescent cancer cell apoptosis can occur, as depletion or inhibition of DYRK1B promotes cell cycle re-entry and increases 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 have the potential to upregulate the c-YES/YAP signaling axis

A cytoplasmic non-receptor protein (c-YES) belonging to the SRC kinase (SFK) family and has been shown to have a function as a biomarker in various types of tumors as well as oncogenic properties [83]. In cancer cells with poor prognosis, c-YES is overexpressed [84, 85]. Some patients treated with EGFR and ALK inhibitors, have c-YES amplification becoming resistant to targeted therapy [86, 87]. Recent studies have shown that, HT29 colon cancer cells, a 5-FU resistant clonal cell population can enter a quiescent G0 state that can revert to the cell cycle when re-exposed when the 5-FU concentration is higher. These quiescent cells showed high levels of c-YES/YAP signal expression. In colon cancer with liver metastases after 5-FU-based neoadjuvant chemotherapy had higher levels of YES1 and YAP transcripts, and also had a positive correlation with shorter patient survival as well as colon cancer recurrence [84].

Recent studies showed that 5-FU induced quiescence of cancer cells could result in high levels of YAP and decreased levels of c-Myc and cyclin E1, which was associated with overall survival and shorter disease-free time [88]. Overall, the

therapeutic targets are thought to have the potential to kill drug-resistant quiescent cancer cells via the c-Yes/YAP signaling pathway.

(e) Quiescent cancer cells have immune evasion capabilities

Metastasis occurs after resection of the primary tumor, through an unknown mechanism with a small number of cancer cells disseminating and persisting as a latent entity. Latent carcinoma cells (LCC) from breast and lung tumors can easily enter a quiescent state in 2% serum low mitogen medium (MLM), whereas the apoptosis marker caspase-3 remains unchanged for one month as a potential entity in the corresponding organs. These quiescent LCC cells still have tumorigenic potential and can induce metastasis. Further studies revealed that this QCC led to escape from immune surveillance and downregulation of NK cell activators via DKK1 protein expression [89].

These findings suggest that quiescent metastases can trigger immunological elimination of latent metastases through selective reactivation of NK cell ligands in cells. Recent research also revealed that in breast tumor cells, QCCs overexpress the silent marker p27 and are able to resist T cell attack by establishing an immunosuppressive niche. QCC associated genes with chemoresistance such as Kdm5a, Kdm5b, Car9,hypoxia (Hif1a), and glucose transporter including 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. These studies suggest that QCC can induce immunotherapy resistance by creating a hypoxic immunosuppressive environment locally to block T cell functionand restore T cell function to eliminate QCC so as to counter immunotherapy resistance [91].

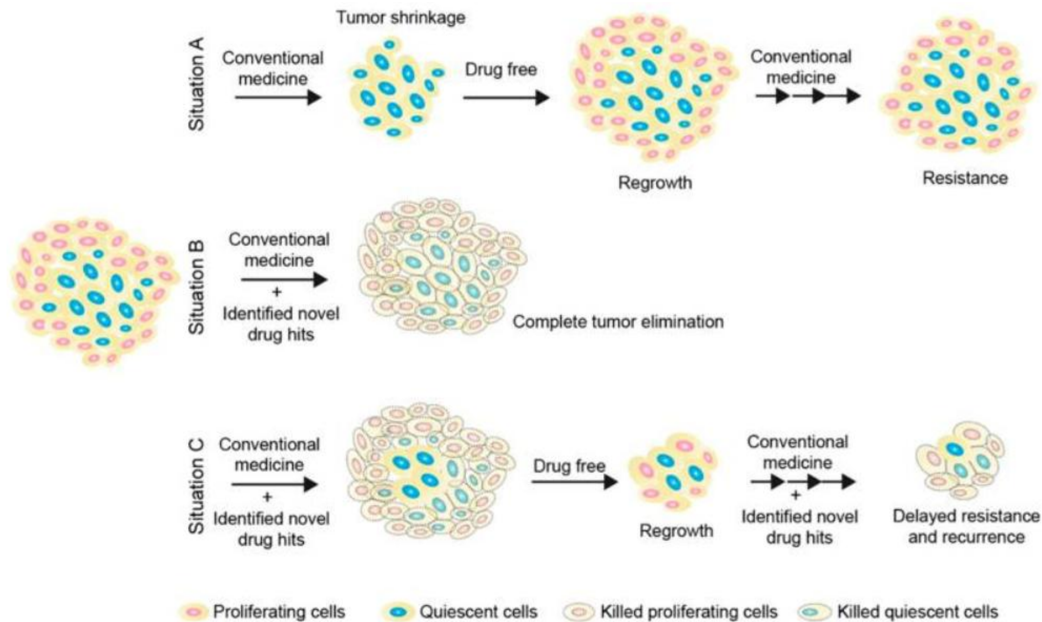

Figure 5. Elimination of tumor cells by targeting both proliferative and quiescent cancer cell populations.

Conclusion

QCC have the ability to evade most cancer treatments and have been associated with resistance to stem cell cancer as well as recurrence. QCC can re-enter the proliferation phase when tumor environmental conditions are favorable. Accumulating research aimed at finding QCC therapy options in cancer cells has revealed several clues to overcome resistance and recurrence of cancer therapy. Here, we review and discuss recent research advances in QCC treatment including reactivating quiescent cancer cells and eliminating them through cell cycle-dependent anticancer reagents, modulating the switch from quiescence to proliferation; and eliminate QCC by targeting its unique features.

Currently, there are many obstacles in cancer treatment to face QCC. Further research is still needed to understand the characteristics of QCC, the regulatory mechanisms of proliferative and stationary phase transitions in cancer cells, and discover new strategies to eliminate QCC in solid cancer cells. This is a long-term challenge and still requires further research in the future.

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