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REVIEW

Chemoquiescence for Ideal Cancer Treatment and Prevention: Where Are We Now?

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Cellular quiescence is a state of reversible cell cycle arrest and is associated with a low metabolic state featured with decreased glycolysis, reduced translation rates, and activation of autophagy, fundamentally to provide nutrients for cell survival similar as seen in hybernation. As signal for quiescence, inactivating the target of rapamycin kinase and resulting reduced cell growth and biosynthesis are essential, but cellular quiescence is not always associated with reduced metabolism since it is also possible to achieve a state of cellular quiescence in which glucose uptake, glycolysis and flux through central carbon metabolism are not reduced. However, in cancer cells, overcoming intrinsic and acquired resistance of cancer stem or cancer dormancy cells to current clinical treatments can be reversed with the acquisition of chemoquiesence. The development of new drug combinations or strategy to treat the highly aggressive and metastatic cancers including relapsed leukaemias, melanoma and head and neck, brain, lung, breast, ovary, prostate, pancreas as well as gastrointestinal cancers which remain incurable in the clinic in spite of aggressive therapies, can be accelerated with the introduction of chemoquiescence agent, for which cancer stem cells or tumor dormancy should be eradicated or removed. Recently potential applications of metformin or chloroquine as well as the potential drugs under investigation such as proton pump inhibitor, sonic hedgehog inhibitor, and Akt inhibitor, are actively investigated in this field of chemoquiescence to achieve cancer cure far beyond those of chemoprevention. In this review article, the evolving concept of chemoquiescence or cancer dormancy will be introduced accompanied by a description of novel target drug development.

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Key Words: Quiescence, Chemoprevention, Cancer stem cells, Cancer dormancy

INTRODUCTION

The late stage cancers, once diagnosed lately, are featured with a high rate of recurrence after primary treatment with the conventional cancer therapies including surgery, radiotherapy, and systemic chemotherapy, finally leading to the death of patients. Therefore, the establishment of the molecular key events underlying cancer initiation and progression before invasive and metastatic diseases is of major interest in basic cancer research as well as in cancer clinic, sincerely hoping to develop new effective clinical therapeutics ablating cancer origin as well as cells responsible for recurrence. As much as newer therapeutic strategies consist of molecular targeting of oncogenic signaling elements that activated in the cancer progenitor cells, and tumor microenvironment prerequisite for cancer progression. The development of chemoquiescence agents getting rid of cancer stem cells (CSCs) or tumor initiating cells (TICs) or keeping tumor dormancy state will improve the efficacy of current therapeutic treatments through the prevention of cancer recurrence and prolongation of patient survival as well as overcoming the limitation of current chemotherapeutics.[1,2] Recent development of cancer biology further defined cancer cells as TICs implicated in primary tumor growth, small subpopulation of highly tumorigenic cells as CSCs causing treatment resistance and disease relapse, migrating cancer stem/progenitor cells as metastasis-initiating cells, and tumor dormancy cells.[2] The malignant

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transformation of multi-potent, tissue-resident, multi-lineagic adult stem cells (SCs) into CSCs endowed tumors with a high self-renewal capacity and aggressive characteristics, providing critical roles for primary tumor growth, metastases, chemo- and radio-resistance, as well as cancer recurrence.[3] Recently, another 2 concepts are put forward in cancer management beyond chemoprevention, that is, tumor dormancy and chemoquiescence. Tumor dormancy represents an important mechanism underlying the observed failure of existing therapeutic modalities to fully eradicate cancers or tumor dormancy might also critically contribute to early stages of tumor development and the formation of clinically undetectable micro-metastatic foci. Though striking parallels exist between the concept of tumor dormancy and CSC theory of tumor propagation, CSC hypothesis similarly predicts that a subset of self-renewing cancer cells-that is CSCs-is responsible for tumor initiation, bears the preferential ability to survive tumor therapy, and persists long-term to ultimately cause delayed cancer recurrence and metastatic progression. However the dormant state of a tumor can also govern CSC behavior, including cell cycle modifications, alteration of angiogenic processes, and modulation of antitumor immune responses. As the latter, the quiescence and immune escape are emerging hallmark features of at least some CSCs, indicating significant overlap between dormant cancer populations and CSCs.[4] In this review article, we explain how CSC or tumor dormancy is important in cancer treatment as well as cancer prevention and describe the possibility of chemoquiescence through the development of novel therapeutic avenues.

MAKE CANCER STEM CELLS IN QUIESCENCE, WHERE ARE WE NOW?

Subsets of mammalian adult stem cells reside in the quiescent state for prolonged periods of time and quiescence of CSCs is also critical to ensure life-long tissue maintenance.[5] Recent advances in SC biology have provided insights into the epigenetic, transcriptional and post-transcriptional control of quiescence and suggested that quiescence is an actively maintained state in which signaling pathways involved in maintaining a poised state are activated. As SCs in adult organs continue to be identified and characterized, their survival, quiescence, and activation depend on specific signals of their microenvironments.[6] Although adult SCs of diverse tissues differ by their developmental origin, cycling activity, and regenerative capacity, it appears that conserved similarities regarding the cellular and molecular components of the SC niche exist. Many organs house both slow-cycling and fast-cycling SC populations, which rely on the coexistence of quiescent and inductive niches for proper regulation.[7] In reality, Campos et al.[8] showed that self-renewal potential of individual cells is partitioned asymmetrically between daughter cells in a robust and cell line-specific fashion, yielding populations of fast- and slow-cycling cells, which differ in their expressions of cell cycle-associated transcripts. While the majority of the cancer cells have a limited ability to divide, a population of CSCs that have the exclusive ability to extensively proliferate and form new tumors can be identified based on marker expression. Growing evidence suggests that pathways that regulate the self-renewal of normal stem cells are deregulated in CSCs, extremely resulting in the continuous expansion of self-renewing cancer cells and tumor formation. Therefore, agents that target the defective self-renewal pathways in cancer cells might lead to improved outcomes in the treatment.[9] Specific signaling pathways play a functional role in CSC self renewal and differentiation and early studies indicate that CSCs are associated with a microenvironmental niche. Thus, the biological properties of CSCs are just beginning to be revealed, and the continuation of these studies should lead to the development of CSC-targeted therapies for further effective cancer treatment.[10] Metastatic dissemination with subsequent clinical outgrowth leads to the greatest part of morbidity and mortality from most solid tumors. Even more daunting is that many of these metastatic deposits silently lie undetected, recurring years to decades after primary tumor extirpation even by surgery or radiation. So, there is also a "metastatic dormancy." As primary tumors are frequently curable, a critical focus now turns to preventing the lethal emergence from metastatic dormancy. Though current carcinoma treatments include adjuvant therapy intended to kill the metastatic tumor cells, since such standard therapies mainly kill cycling cells, this approach carries an implicit assumption that metastatic cells are in the mitogenic cycle. Therefore, the pivotal question whether clinically occult micrometastasis survives in a state of balanced proliferation and death or whether these cells undergo at least long periods of quiescence marked by cell-cycle arrest arises.[11] The treatment implications are thus obvious that if the carcinoma cells are cycling then therapies should target cycling cells, whereas if cells are quiescent then therapies should either maintain dormancy or be toxic to dormant cells. Because this distinction is paramount to rational therapeutic development and administration, we investigated whether quiescence or balanced proliferation is the most likely etiology underlying tumor dormancy or metastatic dormancy. Recently, a computer simulation study which determined that balanced proliferation is

not the likely driving force and that quiescence most likely participates in metastatic dormancy was developed.[12] In conclusion, a greater emphasis on developing diagnostics and therapeutics for quiescent carcinomas is needed now for the clinical achievement of chemoquiescence beyond current chemoprevention.

THE DORMANCY DILEMMA RELEVANT TO CHEMOQUIESCENCE

Even after the best available treatments for primary tumors, cancer can recur after a long disease-free interval, during which period, cancer cells are believed to lie dormant in either primary or metastatic sites, escaping adjuvant cytotoxic treatments. As exemplified in thyroid or prostate cancer, most of these cancers are probably in dormancy because of the facts that most tumor do not progress in size but there is no idea which tumor stay silent before invasive or metastatic cancer, therefore the big clinical unmet medical needs shed the question whether clinician should remove all cancers in dormancy condition. Unfortunately, little is known about how these cells transition to dormancy or how they are reactivated when cancer progresses or recurs. Yumoko et al.[13] have revealed the influences of tumor microenvironment, or niche, on the regulation of tumor dormancy via the signaling pathways of growth arrest-specific 6, bone morphogenetic protein 7 (BMP7), and transforming growth factor bet-1 (TGF-β1), and that the balance between activation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK)[14] MAPK plays a pivotal role in tumor dormancy. Though cancer dormancy is yet a poorly understood stage of cancer progression, the ability to control tumor dormancy can offer novel therapeutic opportunities based on their investigations. Ranganathan et al.[15] also suggested that imbalances in the activity ratio of ERK to p38 MAPK signaling may determine the fate, whether to be tumorigenic or to be dormant and explored that dormancy of tumor cells may be the result of a selective adaptive response that allows disseminated tumor cells to pause their growth and cope with stress signaling imposed through dissemination until growth can be restored. In clinic, tumor cell dormancy may help to explain treatment resistance and recurrence or metastatic reactivation because understanding the dormant stage of tumor cells may help in discovering ways to maintain the dormant state or permanently eliminate dormant residual disseminated tumor cells. Over the past decade, numerous studies indicated that various mechanisms of tumor dormancy exist, including cellular dormancy, angiogenic dormancy, and immunologic dormancy, all as cancer cell quiescence.[16] Regarding metastatic dormancy, metastatic cells undergoing reactivation are nursed by specialized extracellular matrix niches, which support positive signals, such as Wnt and Notch, and attenuate negative signals such as BMP.[17,18] For example, the dormant breast cells can keep quiescence state because of lack of proliferation-stimulating adhesion signaling transduced by the extracellular matrix, the surface receptor urokinase plasminogen activator receptor (uPAR) and α5β1 integrin or the presence of inhibitory signals, such as BMP, from the microenvironment. With the reactivation of dormant cells, fibronectin or the uPAR-integrin α5β1 complex is increased and leads to activation of ERK and inactivation of p38, thus reducing stress signaling and favoring growth. Moreover, gain of expression of COCO, a inhibitory effect of BMP on the Wnt signaling pathway, enables the outgrowth of dormant mammary tumor cells to form metastases in the lung. Subsequently, stroma-derived periostin interacts and thus recruits Wnt ligands while Tenascin C secreted by tumor cell or stromal cell stimulates Notch and Wnt signaling in the dormant tumor cells.[17] Wells et al.[12] published study which determined that balanced proliferation is not the likely driving force and that quiescence most likely participates in metastatic dormancy. In conclusion, since dormancy is a state of quiescent CSC, which are more resistant to chemotherapy and targeted therapy[19] and as this deregulation leads to cross-resistance between the immune response and cytotoxic drugs, the long-term selection that occurs in vivo during tumor dormancy may ultimately result in resistant relapse. Therefore, long-term selection of cancer cells in vitro using tyrosine kinase inhibitors selects cells that harbor the same resistance mechanisms as dormant tumor cells.[20] In near future, a framework to understand the logic of metastatic dormancy and reactivation will also open new avenues for therapeutic intervention.[18,21] Just like standard therapies mainly kill continuously cycling cells, metastatic cells are also in the mitogenic cycle, imparting the pivotal question whether clinically occult micrometastases survive in a state of balanced proliferation and death, or whether these cells undergo at least long periods of quiescence marked by cell-cycle arrest.

SIDE POPULATION CELLS, TUMOR INITIATING CELLS, AND CANCER STEM CELLS

The facts that side population (SP) and CSCs drive and maintain many types of human malignancies and are responsible for being highly resistant to chemo- and radio-therapy have pioneered the possibility of specifically targeting CSCs and SP cells by

exploiting specific pathways involved in drug resistance, or forcing these cells to proliferate and differentiate, thus converting them into a target of conventional therapies.[22] Recent finding suggesting reactive oxygen species related genes as one of the core mechanisms by which CSCs can generate these resistances yields a hope towards the creation of new therapeutic avenues.[23] Additionally the cellular origin of these CSCs whether they have originated from SCs that have lost the ability to regulate proliferation or from more differentiated population of progenitor cells that have acquired abilities to self-renew is still unclear. These are cells responsible for tumor development, progression and response to therapy and relapse, named as CSCs or TICs, featured with cell surface markers such as CD44, CD24,[24,25] and CD133[26] as well as a regulatory network consisting of microRNAs and Wnt/β-catenin, Notch, and Hh signaling pathways.[27,28] Rather than a distinct entity, these cells seem to possess a dynamic status that can be continually dedifferentiated from progenitor or differentiated cancer cells. Therefore, elucidation of this bidirectional transition mechanism would help to perfect the CSC/TIC theory and would be of great value in the development of more effective anti-cancer drugs.[29] SP cells also possess some intrinsic SC properties as they generate non-SP cells in vivo, expressing some "stemness" genes, including Notch-1 and β catenin. The SP phenotype is mainly mediated by ATP-binding cassette (ABC)G2, an ABC half-transporter associated with multidrug resistance (MDR). Patrawala et al.[30] found that the SP is enriched with tumorigenic stem-like cancer cells, ABCG2 expression identifies mainly fast-cycling tumor progenitors, and the ABCG2-population contains primitive stem-like cells. Therefore targeting SP could improve cancer therapy by blocking these transporters.[31] In conclusion, better characterization of SP and CSCs will advance our understanding of SCs and provide us an accessible target for DR in cancer therapy as well as achieving chemoquiescence.

CHEMOQUIESCENCE AS ULTIMATELY TARGET PREREQUISITE FOR IDEAL CANCER PREVENTION

Since cancer stem-like cells are relatively chemoresistant or radioresistant owing to different intrinsic and extrinsic factors including quiescence, enhanced DNA repair, up-regulated cell cycle control mechanisms, and increased free-radical scavengers with possession of a powerful microenvironment that enhances cell survival mechanisms such as hypoxia and interaction with stromal elements,[32] agents or strategy to regulate CSCs or tumor dormancy can be ultimate end of cancer treatment, and chemoquiescence achievement. In case of non-cancer cells, adult SC niches also exist. They are characterized by a dichotomy of cycling and quiescent SCs, while the former are responsible for tissue turnover, their quiescent counterparts are thought to become active upon tissue injury thus underlying the regenerative response.[33] As results, quiescence can prevent adult SCs from accumulating mutations thus ensuring a reservoir of unaltered SCs, but in case of damages, awakening quiescent SCs can be another ultimate way of mucosal healing as seen in inflammatory bowel diseases or other tissue damaging conditions.[34] As exemplified in colon cancer, cancers are thought to share a comparable hierarchical structure of adult tissues with pluripotent and self-renewing CSCs giving rise to more differentiated cellular types. Because of their infrequent cycling, quiescent CSCs are usually refractory to therapy as well as promoting tumor dissemination, that is, dormancy and recurrence condition, while normal SCs are essential in healing.[33] However, the balance or switch between quiescence and aberrant quiescence seems to be very important and to be determined since aberrant self-renewal and quiescence contribute to the aggressiveness of cancers,[8] in which contributing factors are angiogenesis,[19,35] immunological environment,[4,36,37] the presence of pre-metastasis niche by the primary tumor, and the formation of a nurturing organ microenvironment for migrating CSCs.[38] Mechanistically, classical properties of normal SCs are strikingly reminiscent of the observed experimental and clinical behavior of metastatic cancer cells including an unlimited capacity for self renewal and a specific "niche" or microenvironment to grow. The use of the stromal cell-derived factor 1 and chemokine receptor 4 axis is prerequisite for migration stimulation in addition to enhanced resistance to apoptosis and an increased capacity for drug resistance.[39,40] In conclusion, since microscopic human cancers, either primary, recurrent or metastatic, can remain in an asymptomatic, non-detectable, and occult state for a long period of time,[41,42] and elucidating these regulatory machineries can be instrumental in identifying novel early cancer biomarkers and providing a rationale for the development of dormancy-promoting tumor therapies. Differentiation therapy and niche targeting including the self-renewal controlling pathways such as Wnt, Notch, Hh, aldehyde dehydrogenase A2 activity and ABC transporters, and blockade of epithelial mesenchymal transition will be discovered for chemoquiescence to prevent cancer in near future.[43]

OPPORTUNITY FOR DRUG DISCOVERY TO ACHIEVE CHEMOQUIESCENCE IN CLINIC

Adult normal SCs are maintained in a quiescent state but are able to exit quiescence and expand rapidly, differentiate in response to stress, and acquire recurrence or resistance to chemotherapeutics through awakening CSCs. Though the quiescent state appears to be necessary for preserving the self-renewal of SCs and is a critical factor in the resistance of CSCs to chemotherapy as well as targeted therapies, very limited knowledge about quiescence mechanisms is available, hindering the advances in targeting the drug-resistant quiescent CSCs populations in the clinic. Our research group has discovered potential candidates to control tumor dormancy or CSCs with the following potential drugs including the antimalarial drug chloroquine (CQ), anti-diabetic drug metformin, Sonic Hedgehog (Shh) inhibitors, cyclopamine and cerulenin, and ABC blockers, proton pump inhibitor (PPI) and acid pump antagonist (APA).[44] An improved understanding of the molecular mechanisms of quiescence in adult SCs is critical for the development of molecularly targeted therapies against quiescent CSCs in different cancers. Li et al.[45] have extended a better understanding of the intrinsic and extrinsic regulatory mechanisms that control SC quiescence as intrinsic regulatory mechanisms such as p53 gene and other intrinsic regulatory mechanisms including the Forkhead-box O (FOXO), hypoxia-inducible factor 1α (HIF-1α), and nuclear factor of activated T cells (NFAT) transcription factors, signaling through ataxia telangiectasia mutated (ATM) and mammalian target of rapamycin (mTOR) and as extrinsic regulatory mechanisms such as angiopoietin-1, TGF-β, BMP, thrombopoietin, N-cadherin, and integrin adhesion receptors. Regarding ABC intervention, Maugeri-Sacca et al.[46] have studied ABC drug transporters accompanied with the activation of PI3K/AKT and Wnt pathways as regulator for chemoquiescence. Since tumor cells are very heterogeneous comprised of rare TICs and abundant non-TICs with TICs and CSCs having the similar ability of self-renewal and proliferation and both being resistant to drugs, it is not clear yet whether CSCs and originate from normal SCs in consequence of genetic and epigenetic changes and by re-differentiation from somatic tumor cells to the stem-like cells.[46] Probably both mechanisms are involved in the origin of CSCs, achievement of chemoquiescence seems to be not easy, but faithful with the principle that one stone kills two birds, multiple stages of therapeutic plan might be prerequisite.[47] Our lab has continuously tried to develop chemoquiescence agents, after Shh inhibitor such as cyclopamine, cerulenin, chloroquine as autophagy inhibitor, and ABC regulator such as PPI and APA as possible chemoquiescence agents, which are under study now. In accordance with achievements by other investigators including *Wnt* and *Notch* inhibitor, tumor protein p53 and phosphatase and tensin homolog on chromosome 10 regulator, and epidermal growth factor receptor and KIT signaling targets as well as angiogenesis inhibitors,[48,49] it is imperative to design new strategies based upon a better understanding of the signaling pathways that control aspects of self-renewal and survival in CSCs in order to identify novel therapeutic targets in these cells for chemoquiescence. Moreover, over the past several years, a tremendous amount of effort has been invested in the development of new drugs such as nanomedicines taking advantage of the "Achilles' heel" of CSCs by targeting cell-surface molecular markers or various signaling pathways.[50,51]

CHEMOQUIESCENCE WITH CHLOROQUINE

Balic et al.,[14] in their recent publication, showed in vitro treatment with the anti-malarial agent, CQ, N'-(7-chloroquinolin-4-yl)-N,N-diethyl-pentane-1,4-diamine which significantly decreased CSCs translating into diminished in vivo tumorigenicity and invasiveness in a large panel of pancreatic cancers. We also have found CQ effectively inhibited tumorsphere formation as well as dose dependent cytotoxicity against formed tumorpshere. Furthermore, in vivo treatment in combination with gemcitabine was capable of more effectively eliminating established tumors and improved overall survival and CQ additionally showed potent inhibition of Hedgehog signaling by decreasing the production of *Smoothened*, translating into a significant reduction in Shh-induced chemotaxis and down- regulation of downstream targets in CSCs and the surrounding stroma. Firat et al.[52] performed triple therapy with chemotherapeutics, irradiation (γIR) and CQ at doses as low as 5 μM to 10 μM which indeed caused strong apoptosis in glioma treatment and concluded that triple combinations of CQ, γIR and a PI3K/Akt pathway inhibitor permit reduction of the CQ dose required to trigger cell death. These astonishing findings, in recognition of the extensive interactions observed in both healthy and diseased cells, emphasized that the three networks including CQ could be merged into a "metabolism-signaling super-network."[53,54] In addition to chemoquiescence agent CQ, metformin is an oral antidiabetic drug and is of emerging interest for cancer prevention which can kill CSCs. Metformin synergistically interacts with the anti-HER2 monoclonal antibody trastuzumab to sup-

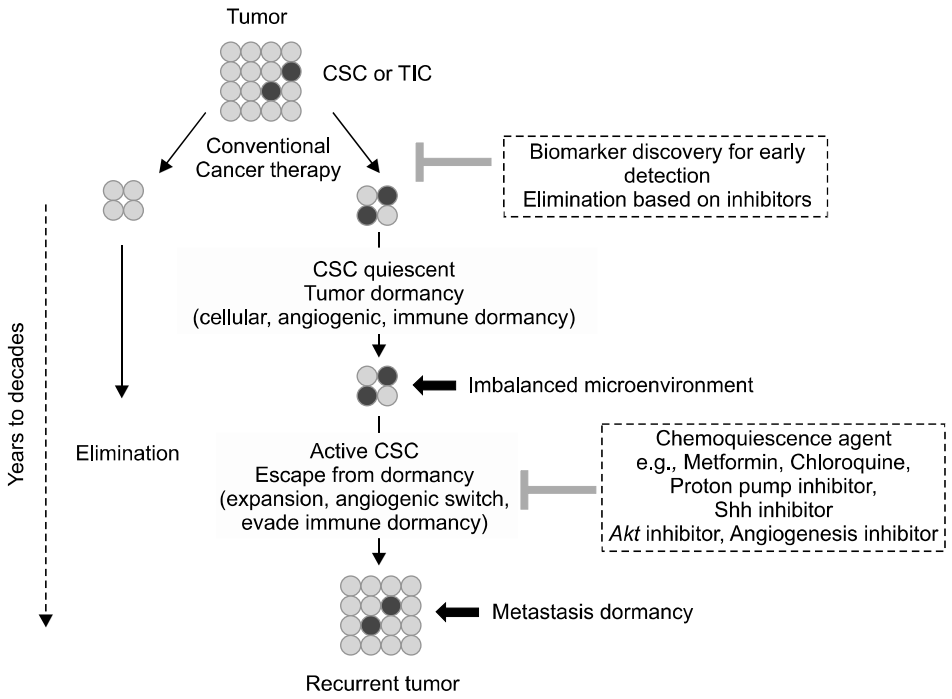

Figure. Chemoquiescence for preventing cancer progression and recurrence. CSC, cancer stem cell; TIC, tumor initiating cell.

press self-renewal and proliferation of cancer stem/progenitor cells in HER2-positive carcinomas.[55] In molecular mechanism, metformin treatment led to a decreased expression of CSC marker genes including CD44, EpCAM, EZH2, Notch-1, Nanog, and Oct4.[56] Moreover, tumor cells are protected from acidic pH by proton pumps.[57] Several studies have demonstrated that the tumor microenvironment of pH 6.5 promoted CSC sphere formation in vitro. PPIs treatment such as esomeprazole (v-ATPase inhibitor) and amiloride (Na^+/H^+ exchanger inhibitor) could inhibit sphere formation.[58]

CONCLUSIONS

Although the concept of "CSC" was first proposed more than a century ago, the first small subpopulations of CSCs were identified from blood mononuclear cells in human acute myeloid leukemia in 1997 and it has attracted a great deal of attention recently owing to advances in SC biology as well as the revisited concept of quiescence or dormancy, since unmet medical needs in cancer treatment are to prevent recurrence as well as overcoming chemo- or radio-resistance. The reason why a small population of cancer cells referred to CSCs have received particular attention, is because CSCs have been shown to be responsible for acquiring stem cell-like properties and becoming the main cause of tumor propagation and metastasis as well as chemotherapeutic or radiation resistance. Though many CSC-targeted therapy methods were expected to cure or prevent cancer by eradicating CSCs as part of chemoquiescence concept, it has not become true in clinic yet. Since the identification of CSC-specific markers, the isolation and characterization of CSCs from malignant tissues, and targeting strategies for the destruction of CSCs might provide a novel opportunity for cancer research, huge efforts are now on progress. Repositioning research for metformin, CQ, PPI, APA, and Shh inhibitor might provide unexpected discovery for chemoquiescence in near future with the expected translational impact of the "old drugs-new uses" repurposing strategy to open a new CSC-targeted chemoprevention era (Figure).[59,60] Ongoing chemopreventive, neoadjuvant and adjuvant trials should definitely establish whether metformin's ability to kill the "dandelion root" beneath the "cancer soil" likely exceeds metformin-related dangers of hormesis.[60] Furthermore, CQ may be one of the most effective and safe sensitizers for cancer therapies based on our and other investigations. Taken together, soon the efficacy of conventional cancer therapies can be dramatically enhanced if used in combination with CQ and its analogs[61-63] thereby achieving chemoquiescence in near future.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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