Pharmacogn Rev. 2020;14(28):155-159
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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Research Article

Thymoquinone: A Potential Therapy against Cancer Stem Cells

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ABSTRACT

Background: Cancer remains to be a major health problem despite advances in treatment. Chemo- and radiotherapy resistance accounting for cancer recurrence have been recently attributed to a subpopulation of cells within the tumor, namely cancer stem cells (CSCs). **Aim:** Hence, it is essential to adopt new therapeutic approaches that target these cells. **Methods and Results:** The black seed extract Thymoquinone (TQ) has shown promising anti-cancer effects on various cancer types. Here, we provide an overview of TQ's potential in targeting CSCs with emphasis on its mechanism of action and shed light on its development as a future drug for cancer therapy. **Conclusion:** TQ showed potency against CSCs either alone or in combination with chemotherapeutic agents.

Key words: Thymoquinone, Cancer Stem Cells, Resistance, Plant-derived drugs, Cancer.

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History

- Submission Date: 19-07-2020;
- Review completed: 03-09-2020;
- Accepted Date: 07-12-2020.

DOI : 10.5530/phrev.2020.14.19

Article Available online
http://www.phcogrev.com/v14/i28

INTRODUCTION

Cancer is a major public health concern globally and the second leading cause of death after myocardial infarction in the United States.[1] Chemotherapy either alone or in combination with other treatments is the most common treatment option in cancer therapy. Unfortunately, chemotherapeutic agents have many adverse side effects and their effectiveness has been greatly limited by drug resistance. Resistance to therapy has been associated with a subpopulation of cells within the tumor, namely cancer stem cells (CSCs). Currently, growing interest is heading towards using compounds from natural sources for cancer treatment, as natural products are less toxic, widely available and cost-effective. Plant-derived drugs have been used traditionally for the treatment of various diseases and scientists are now developing new drugs by combining folk medicine with modern medicine. The plant-derived molecule thymoquinone (TQ) has shown promising anti-cancer activity by inhibiting cancer cell growth and progression *in vitro* and *in vivo*. In this review, we aim to shed light on the potential effect of TQ on CSCs either alone or in combination with other clinically available drugs to achieve enhanced efficacy and overcome resistance to therapy.

Thymoquinone: a naturally derived compound with anti-cancer properties

Thymoquinone is the main active molecule of the essential oil extracted from *Nigella sativa* black seed that has been commonly used as a herbal medicine for the treatment and prevention of a variety of diseases including asthma, diarrhea and dyslipidemia.[2] TQ has a wide range of beneficial biological and pharmacological properties. It possesses outstanding anti-oxidant,[3] hypoglycemic,[4] anti-inflammatory,[5] anti-cancer,[2] neuro-,[6] cardio-,[7] nephro-,[8] and hepato-protective[9] activities. TQ has shown promising effects on various cancer types both *in vitro* and *in vivo*[10] including breast,[11] prostate,[12] gastric,[13] lung,[14] colorectal,[15-18] osteosarcoma[19] and bladder cancer.[20] TQ's anti-cancer mechanism has not been fully understood so far; however, several modes of action have been described. TQ was shown to induce apoptosis in cancer cells by inducing reactive oxygen species, DNA damage, telomere shortening, immunomodulation through inhibition of NF-kappa B (NF-κB) and its regulated gene products and by targeting carcinogenic signaling pathways such as JAK/STAT and PI3K/Akt signaling.[21] TQ was also shown to regulate epithelial to mesenchymal transition and to inhibit cancer metastasis by reducing matrix metalloproteinase (MMP-2 and MMP-9) secretion and the expression of TWIST1.[22,23]

Naturally derived drugs are an important component of combination chemotherapy and are integrated with traditional regimens to improve efficacy, safety and tolerability.[24] They establish their effects by either acting synergistically with conventional drugs or by sensitizing cancer cells to these drugs.[25] TQ was shown to enhance chemotherapeutic potentiality when combined with clinically available drugs.[21] Combination of TQ with 5-Fluorouracil increased apoptotic activity in gastric cancer cells *in vitro* and *in vivo*.[26,27] Kensara *et al.* reported that 5-Fluorouracil and TQ cooperate to repress the expression of pro-cancerous Wnt, β-catenin, NF-*κB*, COX-2, iNOS, VEGF and TBRAS and to up-regulate the expression of anti-tumorigenesis markers DKK-1, CDNK-1A, TGF-β1, TGF-βRII, Smad4 and GPx

Cite this article: Ballout FR, Gali-Muhtasib H. Thymoquinone: A Potential Therapy against Cancer Stem Cells. Pharmacog Rev. 2020;14(28):155-9.

in colorectal carcinogenesis in rats.[28] Combination of cisplatin and TQ was shown to be highly effective in enhancing cisplatin-mediated cytotoxicity in lung and ovarian cancer cells and in mouse models.[29,30] TQ and paclitaxel combination showed synergistic effects against triple negative breast cancer.[31] Treatment with TQ and docetaxel induced cytotoxicity and apoptosis by modulating PI3K-Akt pathway in Castrate-resistant prostate cancer cells.[32] Moreover, TQ in combination with zoledronic acid showed significant synergistic cytotoxic activity and DNA fragmentation in PC-3 and DU-145 prostate cancer cells.[32] In addition to its adjuvant chemotherapeutic effect, TQ also mediates radio-sensitization[33] whereby it was found to exert supra-additive cytotoxic and apoptotic effects on MCF7 and T47D breast cancer cells when combined with a single dose of ionizing radiation (2.5 Gy). TQ was also shown to have protective effects on radiation induced small intestine injury in mice by inhibiting p53 pathway, thus reducing intestinal cell apoptosis.[34] Considering TQ's multiple targeting mechanisms, its potency in small concentrations, *in vivo* efficacy and its effectiveness when combined with chemo and radiotherapy, this compound merits further clinical investigation.

Cancer stem cells

Cancer stem cells (CSCs) are characterized by self-renewal, multipotency, limitless proliferation potential, angiogenic and immune evasion features.[35] Intriguingly, CSCs are relatively highly resistant to conventional therapeutic measures and are thus responsible for tumor relapse due to the expression of DNA repair mechanisms, detoxifying enzymes, anti-apoptosis proteins and multiple drug resistance transporters.[36,37]

Populations of CSCs have been identified and isolated from various cancer types using a combination of surface markers including CD24, CD44, CD133, EpCAM, lgr5, among others.[38] CSCs reside in a tumor-promoting microenvironment.[39] Genetic or epigenetic aberrations in the stem cells compartment may lead to alterations of the tumorigenic niche[40,41] that is composed of transformed myofibroblasts, recruited myeloid cells and extracellular components producing hepatocyte growth factor (HGF), tumor necrosis factor α (TNF-α) and interleukin (IL)-6, which promote dedifferentiation, carcinogenesis and invasiveness.[41,42]

Evidence suggests that it is the fine tuning between pathways involved in self-renewal that switch a normal stem cell into a malignant stem cell.[43] Multiple signaling pathways are involved in CSCs survival, maintenance and self-renewal. Key stemness-signaling pathways include Wnt/β-catenin, JAK/STAT, Hedgehog, Notch and PI3K/Akt (Figure 1).[44]

Wnt signaling pathway is involved in embryonic development and homeostasis of tissues. Mutations in the APC gene, β-catenin, or the regulatory proteins in the Wnt pathway result in constant activation.[45] This may lead to uncontrolled proliferation, a shift from asymmetrical to symmetrical divisions and augmented survival. Wnt signaling is also involved in the process of epithelial to mesenchymal transition (EMT), invasion and self-renewal or cancer cell dedifferentiation into CSCs.[42,46]

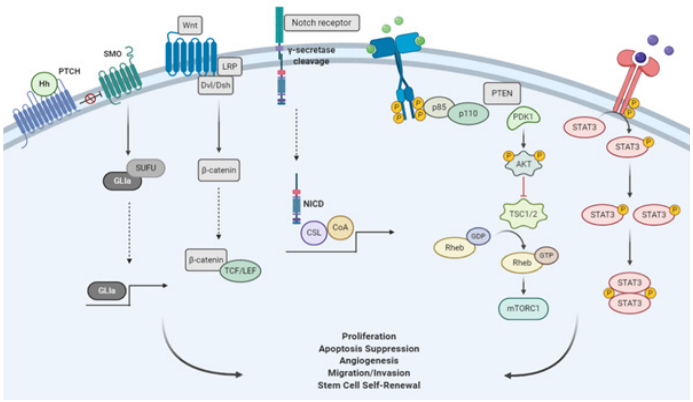

Figure 1: Signaling pathways involved in CSCs survival, maintenance and self-renewal.

Studies have shown that STAT3 signaling, which in normal cells is involved in physiological functions including development, differentiation, immunity and metabolism, is constitutively active in cancer stem cells. Over activation of STAT3 in CSCs may be critical for maintaining their stemness by increasing expression of genes such as c-Myc and β-catenin, the ability to self-renew and differentiate[47] and may promote tumorigenesis, metastasis and recurrence.[47,48] In addition, overactivation of STAT3 in CSCs may generate an inflammatory positive feedback loop[49] in which STAT3 promotes production of proinflammatory cytokines, notably IL-6 that in turn stimulates STAT3 activation. This inflammatory feedback loop can promote tumor progression.

Notch signaling has been reported to promote the self-renewal of CSC in several malignancies and to participate in tumor–stroma and tumor–endothelium interactions in CSC niches in primary and metastatic tumors.[50] Notch signaling regulates both the formation of CSCs and the acquisition of the EMT phenotype by cross talking with several transcription and growth factors relevant to EMT such as Snail, Slug and TGF-β,[51] which are associated with drug resistance. Inappropriate Notch activation stimulates proliferation, restricts differentiation and/or prevents apoptosis. Several classes of Notch inhibitors have been developed to reverse EMT and stemness in CSCs. The strongest evidence for the role of Notch in CSC is in breast cancer, embryonal brain tumors and gliomas.[51]

The Hedgehog (HH) pathway is involved in embryogenesis, adult tissue homeostasis and repair, regulation of the epithelial-to-mesenchymal transition and the control of cell survival and proliferation.[52] Recently, the HH pathway has been shown to be involved in the regulation of proliferation, maintenance and self-renewal capacity of CSCs.[53]

The PI3K/Akt/mTOR signaling pathway is crucial for cell proliferation, angiogenesis, metabolism, differentiation and survival and is frequently improperly regulated in most human cancers.[54] Recent studies have provided evidence for the importance of this pathway in maintaining the CSCs population through induction of EMT, regulation of surface markers like CD133 and EpCAM and regulation of ATP binding cassette transporters (ABCG2) activity.[53]

Another mechanism of CSCs resistance is evading apoptosis. This is mediated through various mechanisms including impaired apoptotic machinery, increased DNA damage repair, altered cell cycle checkpoint control and upregulation of MDR proteins.[55] Upregulation of anti-apoptotic proteins such as cFLIPS and inhibitors of apoptosis proteins (IAPs) and dysregulation of Bcl-2 family members were shown to be associated with the survival of CSCs.[44] Furthermore, production of interleukin-4 (IL-4)[56] and activated NF-κB[57] could protect CSCs from apoptosis.

Collectively, dysregulation of these pathways contributes to CSC resistance to chemotherapy and radiotherapy and to cancer recurrence and metastasis.

Cancer stem cells and the promise of TQ

Direct CSC targeting can be achieved by several approaches which include inhibiting self-renewal pathways including Wnt, Notch and Hedgehog, as well as selectively targeting surface markers, inhibiting ABC cassette, interfering with vital anti-apoptotic or metabolic pathways, activating differentiation pathways and/or by acting on the protective microenvironment (Figure 2).[55,58] Recently, much attention has focused

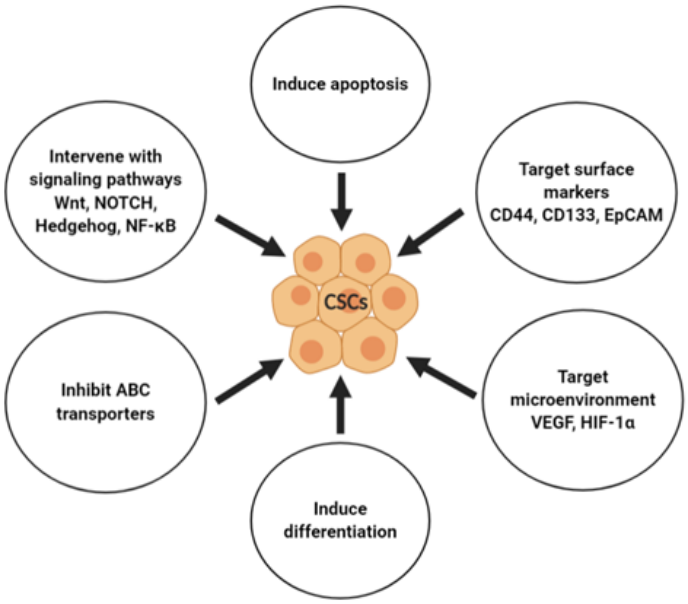

Figure 2: Drug-induced mechanisms for targeting cancer stem cells (CSCs).

on several phytochemicals showing promising anti-cancer properties due to their safety, availability, cost effectiveness and more importantly their ability to improve efficacy when combined with conventional chemo- and radiotherapy.[24] Since CSCs are more resistant to conventional therapies in comparison with the differentiated cells constituting the tumor bulk, a combination of naturally derived drugs and conventional anti-cancer drug therapies may have the potential to overcome tumor resistance and reduce recurrence . TQ's evident potency against various cancers has increased researchers' interest in investigating its effect on CSCs either alone or in combination with other clinically available drugs. Studies are still limited; however, the findings hold great promise. We have recently shown that TQ targets an enriched population of 5-flurouracil sensitive and resistant colorectal CSCs both *in vitro* and *in vivo*. We employed a 3D sphere formation assay to enrich for colorectal CSCs from HCT116 human colorectal cancer cells. TQ treatment inhibited sphere forming ability, reduced cellular proliferation and down regulated the expression of CD44 and EpCAM surface markers and induced apoptosis and DNA damage in colon spheres both *in vitro* and *in vivo* (Ballout *et al.* 2020 in press). Another study by Ndreshkjana *et al.* (2019)[59] has recently reported that the combination of 5-flurouracil and TQ and their hybridization through esterification (SARB hybrid) targets stem cell gene signature in colorectal cancer cells by downregulating two key stem cell regulatory pathways, WNT/β-catenin and PI3K/AKT pathways.[59] In addition to colorectal cancer, TQ was shown to target stemness in human renal carcinoma cells by suppressing the cell sphere formation and the expression of aldehyde dehydrogenase, Nanog, Nestin, CD44 and Oct-4.[60] TQ and gemcitabine combination depleted breast cancer-associated stem cell ($CD44^{(+)}/CD24^{(-)/(low)}$) clone within MCF-7 and T47D breast cancer cells.[61] Similarly, TQ was shown to enhance paclitaxel anti-cancer activity and to sensitize breast cancer cells through the depletion of breast cancer-associated stem cell clone ($CD44^{+}/CD24^{-}$) in both MCF-7 and T47D cells.[62] Therefore, traditional chemo-radiotherapy should be combined with new practical therapeutic approaches that target CSCs and prevent relapse.[63,64]

A major limitation for TQ's clinical translation lies in its hydrophobicity, poor bioavailability and high capacity to bind to plasma proteins.[65] TQ nanoparticle encapsulation could serve as a new platform for overcoming these limitations, thus promoting clinical testing of TQ. So far, several TQ nanoparticle formulations including polymeric, liposomal and solid lipid nanoparticles have been tested against colon, prostate, cervical and breast cancer, as well as leukemia and multiple myeloma.[66-69] A recent study by Ibiyeye and Zuki (2020)[70] showed that combined doxorubicin/thymoquinone-loaded cockle-shell-derived aragonite calcium carbonate nanoparticles can efficiently target breast CSCs by enhancing apoptosis, reducing ALDH activity and decreasing the expression of CD44 and CD24 surface makers. This combination regimen also reduced cellular migration and invasion and inhibited 3D sphere formation by distorting sphere architecture when compared to the free drugs and the single drug-loaded nanoparticle.[70]

Proposed mechanism of action of TQ for targeting CSCs

Few studies have reported the effect of TQ on CSCs and little is known about its mechanism of action against these cells. The mechanism of TQ action on several types of cancer is not yet fully understood; however, several modes of action have been described that could also explain its promising potential against CSCs population.

As previously discussed, TQ possess an ability of multilateral targeting of various cellular and molecular signaling pathways dysregulated in cancer. TQ was shown to regulate self-renewal associated signaling pathways, which are crucial for CSCs survival and for evading apoptosis. TQ was shown to inactivate the JAK/STAT signaling pathway by inhibiting STAT3 phosphorylation, reducing c-Src and JAK2 activity and by attenuating the expression of STAT3 target gene products.[71] TQ is known to modulate Wnt signaling through GSK-3β activation, β-catenin translocation and reduction of nuclear c-myc.[72] TQ was demonstrated to down regulate NF-*κB* and inhibit signaling through PI3/AKT pathway.[73,74] Furthermore, TQ induced apoptosis through activation of p53, induction of Bax, PARP and caspase 3 cleavage, downregulation of Bcl-2 and XIAP and induction of reactive oxygen species (reviewed in.[75] TQ also inhibits epithelial to mesenchymal transition by reducing matrix metalloproteinase (MMP-2 and MMP-9) secretion and the expression of VEGF and TWIST1.[22-23]

Most pathways targeted by TQ are involved in CSCs maintenance and death resistance; thus, TQ is a compound that could possibly inhibit CSCs populations in tumors (Figure 3) which emphasizes the need for its in-depth clinical investigation.

Figure 3: An overview of TQ's mechanism of action against CSCs.

CONCLUSION

Ample evidence has associated cancer recurrence and resistance to therapy to a population of CSCs. In recent years, many studies have revealed the anti-cancer potential of TQ and its ability to modulate various signaling pathways that are aberrantly regulated in cancer. Here, we summarized the current state of TQ's potential in targeting CSCs in various cancer types and focused on its mechanism of action. TQ's potency against CSCs either alone or in combination with

chemotherapeutic drugs may provide a potential curative strategy for the management of cancer recurrence and overcoming aggravating therapy resistance. TQ nanoparticle encapsulations are becoming more clinically attractive because of their improved bioavailability, delivery and targeting capacity. Assessing the efficacy of such nanoparticles in combination with conventional chemotherapy holds promise for achieving effective treatment strategies that specifically target the CSC population and sensitize tumor tissues to treatment.

ACKNOWLEDGEMENT

The schematic illustrations were created using Biorender.com.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CSCs: Cancer Stem Cells; **EMT:** Epithelial-Mesenchymal Transition; **EpCAM:** Epithelial cell adhesion molecule; **TQ:** Thymoquinone; **NF-κB:** Nuclear factor kappa; **MMP:** Matrix metalloproteinase; **VEGF:** Vascular Endothelial Cell Growth Factor; **COX-2:** Cyclo-oxygenase 2; **TGF-β:** Transforming Growth Factor-Beta; **Lgr5:** Leucine-Rich Repeat-Containing G-Protein-Coupled Receptor; **IL-4:** Interleukin 4; **IL-6:** Interleukin 6; **APC:** Adenomatous polyposis coli; **ABCG2:** ATP binding cassette transporters; **HH:** Hedgehog; **MDR:** Multi-drug resistance; **ALDH:** Aldehyde dehydrogenase; **3D:** Three-dimension; **Bcl-2:** B-cell lymphoma 2; **GSK-3β:** Glycogen synthase kinase 3β; **PPAR:** Peroxisome proliferator-activated receptor; **XIAP:** X-Linked Inhibitor of Apoptosis Protein.

SUMMARY

This paper reviews the most recent findings on Thymoquinone's potential in targeting Cancer Stem Cells with a focus on its mechanism of action. Cancer Stem Cells are resistant to therapy and associated with tumor relapse. Thymoquinone targets chemo-resistant Cancer Stem Cells and combining Thymoquinone with conventional therapy holds promise in preventing tumor relapse.

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Cite this article: Ballout FR, Gali-Muhtasib H. Thymoquinone: A Potential Therapy against Cancer Stem Cells. Pharmacog Rev. 2020;14(28):155-9.