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Review

Modulation of diverse oncogenic transcription factors by thymoquinone, an essential oil compound isolated from the seeds of *Nigella sativa* Linn

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ABSTRACT

Thymoquinone (TQ), isolated almost fifty years ago, is the main bioactive constituent of black seed essential oil extracted from the seed of *Nigella sativa*. TQ has been shown to have promising effects against a variety of inflammatory diseases and cancer. Cancer development is a multistep process where normal cells acquire qualities that enable the cells to proliferate continuously and migrate to distant sites in the human body. Drugs that interfere with this process are considered potential anti-cancer therapeutics, which may ultimately result in their clinical usage. TQ is once such compound which has been reported to modulate several major signaling pathways and key oncogenic molecules that play a prominent role in cancer initiation, progression, invasion, metastasis, and angiogenesis. Various studies have reported that TQ can enhance the anti-cancer potential when co-administered with several chemotherapeutic agents while reducing their toxic side effects. In addition, TQ has been shown to inhibit the growth of breast, prostate, pancreatic, colon, lung, and hematological malignancies in different mouse models of cancer. This review focuses on TQ's chemical and pharmacological properties, its diverse molecular targets and also provides clear evidence on its promising potential under preclinical and clinical settings.

Contents

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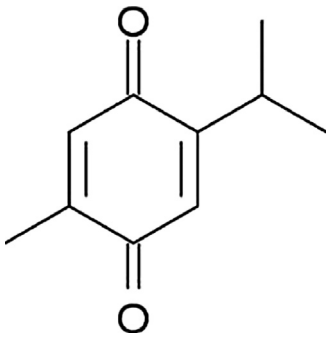

Fig. 1. Structure of thymoquinone.

Table 1
Physiochemical properties of TQ.

IUPAC name	2-Isopropyl-5-methyl-1,4-benzoquinone
Molecular formula	$C_{10}H_{12}O_2$
Molar mass	164.204 g/mol
Appearance	Crystalline and dark yellow
Melting point	44–45 °C
CAS number	490–91-5
PubChem CID	10281

1. Introduction

Several lines of evidence suggest that natural product derived compounds play a pivotal role in the drug discovery and development processes [1–5]. Nature has continued to influence the design of small molecules and their derivatives, thus providing novel molecules with profound biological activities ranging from alleviating pain, inflammation associated diseases, and cancer [3,5–8]. One such compound is thymoquinone (TQ) (Fig. 1), a volatile oil compound isolated from the seeds of *Nigella sativa* Linn. *Nigella sativa* (NS) is commonly known as the black cumin seed and belongs to the botanical family of Ranunculaceae. In the Middle East, NS is commonly known as *Habbatul Baraka* or the "seed of blessing" because it has curative potential as described in the Old Testament and by the prophet Muhammad himself [9,10]. For over 2000 years, black cumin seeds have been used in traditional MiddleEastern medicine as a natural remedy for bronchial asthma and eczema [11,12]. TQ was first isolated from black cumin seeds [9], and was also found to be ubiquitously present in the essential oils from the leaf extracts of several *Origanum* species (0.04 and 23.7%) [13], in the fresh aerial parts of *Eupatorium ayapana* [14], in the heartwood essential oils of *Calocedrus decurrens* [15], the aerial flowering parts of *Thymus vulgaris L.* [16], and from *Nepeta distans Raul* [17]. NS seed extract is composed of >30% w/w fixed oils and 0.4–0.5% of volatile oil [18]. The NS seed extract has several bioactive components such as TQ, dithymoquinone (Nigellone), thymol, carvacrol, nigellicine, nigellidine, and α-hederin, which have demonstrated potential cytotoxic and anticancer properties [19–29]. Interestingly, α-hederin, a pentacyclic triterpene saponin found in NS seeds, has been shown to exert potent anticancer effects *in vitro* and *in vivo* [30–35].

TQ is the most bioactive volatile oil component of the NS seed extract and constitutes 18.4–24% [36]. TQ exists as a keto form (a ketone or an aldehyde), enol form (an alcohol), as well as mixtures of these. Of these, the tautomeric keto form is the major variety and is responsible for its biological activities (Table 1) [37]. Recent pharmacological investigations of the black cumin seed extract have shown wide-ranging biological activities such as being anti-inflammatory [38], and it has been evaluated in pre-clinical and clinical settings [39,40]. Several recent studies have reported that besides its anti-inflammatory activity, TQ is anti-microbial, effective in rheumatoid arthritis, multiple sclerosis, neurological disorders, atherosclerosis, osteoporosis, hypertension, and epilepsy [37,41–45]. Several studies have reported that TQ suppresses proliferation of a variety of cancer cells, including breast adenocarcinoma, colorectal carcinoma, leukemia, osteosarcoma, ovarian carcinoma, and pancreatic carcinoma cells [9,45–54], and minimally toxic to normal cells [19]. TQ is a hydrophobic molecule; therefore, its solubility and *in vivo* bioavailability are major limitations. Therefore, several novel analogs of TQ were synthesized to increase its *in vivo* bioavailability. The molecular micelle modified poly (d,l lactide-co-glycolide) (PLGA) nanoparticles were shown to inhibit the proliferation of MDA-MB-231 breast cancer cells [55]. In addition, TQ loaded liposomes also inhibited the growth of MCF7 and T47D breast cancer cells [56]. Caryophyllyl and germacryl conjugates, as well as fatty acid conjugates, of TQ inhibited the growth of HL-60 human leukemia, multidrug-resistant KB-V1/Vbl cervical carcinoma, 518A2 melanoma, and MCF-7/Topo breast cancer cells and induced apoptosis in these cell lines [57] (Table 2). Several recent reports in the past five years have emphasized the diverse anticancer activities of TQ. In this review, we comprehensively discuss the *in vitro* and *in vivo* anticancer properties of TQ and the various oncogenic transcription factors that modulate molecular and cellular mechanisms such as proliferation, invasion, migration, angiogenesis, and apoptosis.

2. Transcription factors

In normal cells, transcription of genes is a highly regulated process that is critical for normal cell development and differentiation. Transcription factors are important biological molecules that regulate various cellular responses to external signals. They play a very important part in the initiation of a response to an acute or chronic insult to the cells. For example, upon exposure to cytokines, such as tumor necrosis factor (TNF) or interleukin 6 (IL) or epidermal growth factors (EGF), there is cellular activation of several kinases, which ultimately leads to the activation of nuclear factor κB (NF-κB) or signal transducer and activator of transcription (STAT) transcription factors. In cancer cells, constitutive activation of pro-survival transcription factors often leads to uncontrolled cell proliferation, thereby playing a critical role in tumor initiation and progression [58–61]. There are several oncogenic transcription factors, including NF-κB, STATs, nuclear factor (erythroid-derived 2)-like 2 (Nrf-2), fork head box protein M1 (FoxM1), peroxisome proliferator associated receptor gamma (PPARγ), hypoxia inducible factor 1α (HIF1α), Wnt/β-catenin, activator protein (AP-1), c-Met (also known as hepatocyte growth factor receptor), and hedgehog (HH/GLI), which are often deregulated in cancer cells. This leads to malignant transformation of cells and resulting in uncontrolled proliferation, anti-apoptosis, distant site metastasis, multi-drug resistance phenotypes, and radioresistance [5,60].

2.1. Nuclear factor κB (NF-κB)

Chronic inflammation and its associated infections account for approximately 20% of cancer related deaths [58,61,62]. Chronic inflammation and its associated infections account for approximately 20% of cancer related deaths [58,61,62]. NF-κB is an acute phase response protein that is able to initiate transcription of a variety of genes involved in the regulation of cell cycle, tumor suppressors, cell survival, proliferation, metastasis, angiogenesis, and apoptosis [63]. NF-κB was first discovered by David Baltimore in 1986 [64]. Under normal physiological conditions, NF-κB is found in the cytoplasm, and upon appropriate activating signal, can translocate to the nucleus and initiate transcription of genes involved in cell survival signaling [65], pro-and anti-inflammatory reactions [66], memory formation [67,68], cell adhesion mainte-

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