

REVIEW

Tocotrienol as a potential anticancer agent

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Vitamin E is composed of two structurally similar compounds: tocopherols (TPs) and tocotrienols (T3). Despite being overshadowed by TP over the past few decades, T3 is now considered to be a promising anticancer agent due to its potent effects against a wide range of cancers. A growing body of evidence suggests that in addition to its antioxidative and pro-apoptotic functions, T3 possesses a number of anticancer properties that make it superior to TP. These include the inhibition of epithelial-to-mesenchymal transitions, the suppression of vascular endothelial growth factor tumor angiogenic pathway and the induction of antitumor immunity. More recently, T3, but not TP, has been shown to have chemosensitization and anti-cancer stem cell effects, further demonstrating the potential of T3 as an effective anticancer therapeutic agent. With most of the previous clinical studies on TP producing disappointing results, research has now focused on testing T3 as the next generation vitamin E for chemoprevention and cancer treatment. This review will summarize recent developments in the understanding of the anticancer effects of T3. We will also discuss current progress in clinical trials involving T3 as an adjuvant to conventional cancer therapy.

Introduction

Tocopherols (TPs) and tocotrienols (T3) belong to the vitamin E family, each composed of four different isomers: alpha, beta, gamma and delta. The chemical structures of TP and T3 are made up of an aromatic chromanol ring and an isoprenoid chain, with T3 containing three unsaturated phytol side chains (1). Both TP and T3 are well-known antioxidants and are potent in scavenging peroxy radicals through their chromanol ring (2,3). Whereas TP is found in the leaves and seeds of most plants, T3 is less abundant, present mainly in palm oil and rice bran. Both TP and T3 are absorbed into the intestine along with dietary fats and are secreted in chylomicrons, entering circulation via the lymphatic system (4). T3 is rapidly cleared from circulating plasma, whereas TP, especially alpha-TP (α -TP), is preferentially secreted into plasma (5). The half-life of α -, gamma- (γ -) and/or delta- (δ -) T3 in plasma ranges from 2–4 h, and it is 4- to 8-fold shorter than the half-life reported for α -TP (6). Thus, among the vitamin E isomers, α -TP is the most common form found in human diets; it also has the highest bioavailability.

Owing to its highest bioavailability, α -TP is the best characterized vitamin E isomer and has been examined in a large number of studies (7). Data from early epidemiological studies suggest that vitamin E consumption may be associated with reduction of prostate cancer incidence. For example, in the Alpha-Tocopherol Beta-Carotene Can-

cer Prevention Study, male smokers who consumed α -TP daily were found to have a 32% decrease in prostate cancer incidence (8). Low intake of vitamin E was also associated with increased rectal cancer incidence in women (9). Despite of these promising data, in two of the largest chemoprevention trials of this century, vitamin E (α -TP to be precise) consumption was found to have no beneficial effect in reducing cancer incidence (10,11). In the Woman Health Study (WHS) trial, women who took α -TP every other day had a similar chance of developing lung, breast and colon cancer as those who took the placebo (10). Similarly, in the Selenium, Vitamin E and Prostate Cancer Chemoprevention Trial (SELECT) that was completed in 2008, α -TP consumption, either alone or in combination with selenium, was found to have no significant effect in reducing prostate cancer incidence (11).

Due to the disappointing results from these two clinical trials, as well as several recent *in vitro* studies that will be discussed in detail in this review, research has now been refocused on the T3 isomers. Here, we will summarize some of the recent data demonstrating the unique anticancer effects of T3. We will discuss how these distinct properties may make T3 a promising chemopreventive and chemotherapeutic agent. Finally, we will highlight the recent progress in the development and testing of T3-based cancer therapies.

Tocotrienols in cancer chemoprevention

Although T3 has not yet been evaluated as a chemopreventive agent in large-scale clinical trials, its chemopreventive properties have been studied extensively in a number of animal models. In contrast to the effects of α -TP in animal carcinogenesis models (colon, prostate, mammary and lung) that often produce inconsistent results (12), most of the studies on T3 have demonstrated positive chemopreventive effects (13–18). However, mechanistic explanations for its unique chemopreventive effect remain hypothetical (Figure 1).

Chemoprevention of T3 in animal tumor models

Two early reports which compared the chemopreventive ability of TP and T3-enriched sources in 7,12-dimethylbenz(a)anthracene-induced rat mammary carcinoma models found that T3 treatment is associated with significantly lower tumor incidence, lower tumor numbers and longer tumor latency (14,15) when compared with the TP treatment and control groups. In a 2-acetylaminofluorene-induced rat hepatocarcinogenesis model, T3 administration was associated with a reduced severity of hepatocarcinogenesis (16) and decelerated liver tumor formation (17,18). In addition, Wada *et al.* (19) demonstrated that oral administration of a tocotrienol-rich fraction (TRF) resulted in a significant reduction of tumor number per mouse in a spontaneous model of liver carcinogenesis. The same group also showed that this TRF can suppress lung tumor formation induced by 4NQO and glycerol (19). Recently, using a transgenic adenocarcinoma mouse prostate model, Barve *et al.* (20) showed that daily supplementation with a TRF for 16 weeks resulted in a lower incidence of prostate tumor formation. The incidence of high-grade neoplastic lesions was also reduced by the same treatment, clearly indicating the chemopreventive effect of T3 consumption (20). Interestingly, a previous report from the same group as well as a recent study (21) demonstrated that γ -TP treatment can also inhibit prostate cancer development (22). The authors suggested that T3 and TP work differently in suppressing prostate carcinogenesis, with TP acting mainly through the upregulation of detoxifying and antioxidant enzymes (22), whereas T3 directly modulates cell cycle regulatory proteins and increases the expression of pro-apoptotic proteins (20). Collectively, T3 appears to possess chemopreventive properties against different types of cancer that deserves further investigation, especially in clinical studies.

Abbreviations: CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; NF, nuclear factor; TP, tocopherol; TRF, tocotrienol-rich fraction; VEGF, vascular endothelial growth factor.

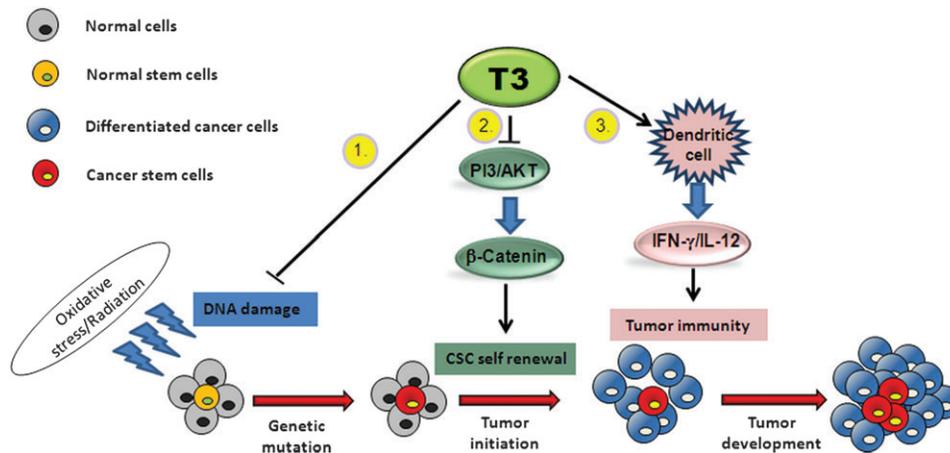


Fig. 1. Chemoprevention by tocotrienol. DNA damage induced by oxidative stress or radiation drives tumor development by inducing genetic mutation, with the CSCs being the tumor initiating population. 1. Tocotrienol inhibits tumor development possibly through blocking oxidative stress or radiation-induced DNA damage. 2. It also suppresses CSCs self-renewal and tumorigenicity through inactivation of the PI3/AKT/ β -catenin pathway. 3. By promoting dendritic cell-mediated tumor immunity, tocotrienol may facilitate the elimination of tumor cells at the early stage of tumor initiation and achieve chemopreventive effect.

Protective role of T3 against oxidative stress-induced DNA damage

Oxidative stress-induced DNA damage is the primary cause of genetic mutation in mammalian cells. Majority of the damaged DNA are detected and repaired by the DNA damage checkpoint but the unrepaired damage will accumulate and eventually lead to cancer development. Recently, T3 has been suggested to play a protective role against oxidative stress-induced DNA damage, which may contribute to its chemopreventive function. Convincing evidence came from a recent animal study by Budin *et al.* (23), which demonstrated that consumption of TRF significantly reduced oxidative DNA damage. The antioxidant effect of T3 appears to play a major role as the decrease in DNA damage was associated with a reduction in the lipid peroxidation by-products (malondialdehyde and 4-hydroxynonenal). Similar results were obtained by the study of Taridi *et al.* (24), which reported a decrease in DNA damage and an improvement in cognitive function in rats that fed with TRF. These findings were consistent with that reported in a randomized clinical trial which demonstrated that consumption of a TRF not only reduced the level of the oxidative stress marker 8-hydroxy-2-deoxyguanosine in urine sample but also significantly suppressed the level of DNA damage in the same human subjects (25).

Notably, the protective effect of T3 is not specific for oxidative stress-induced DNA damage. Using gamma-T3 and delta-T3, respectively, two recent studies reported the protection of hematopoietic progenitor cells from gamma irradiation-induced DNA damage (26,27). The fact that TP, which has similar antioxidative effect as T3, failed to achieve the same radioprotective function suggested that T3 may induce additional protective mechanisms (28). This is supported by the findings that T3, but not TP, regulates the expression of a number of DNA damage response genes (28). Whether these genes are important for the protective effect of T3 against DNA damage remains to be further elucidated.

Modulation of the immune response by T3

Immune surveillance is believed to function as the primary defense against cancer by detecting and destroying tumor cells. Therefore, suppression of the host immune response or loss of tumor immunity has been suggested to promote tumor development. Interestingly, two recent studies demonstrated that T3 supplementation has an immune-promoting effect. A study by Ren *et al.* (7), in which young or old mice were fed with either T3 plus TP or TP alone revealed that lymphocyte proliferation, which was found to be decreased in older mice, was significantly induced by T3 when compared with the control group. This was further confirmed by treating the isolated mouse lymphocytes from old mice with T3 *in vitro*, suggesting that T3 may have beneficial effects against aging-associated impairment

of the immune system. Consistent with these observations, a recently completed clinical trial also reported that oral consumption of T3 has an immunostimulatory effect (29). This study revealed that in healthy females who consumed 400 mg of a TRF daily for 2 months, antibody production after immunization with tetanus toxoid was significantly upregulated when compared with the placebo group, suggesting that T3 treatment enhanced the immune response to tetanus toxoid vaccination. However, in a separate study by the same research group, neither TRF nor TP supplementation were found to affect the immune parameters of healthy individuals (30), although the use of lower T3 concentrations in this study may account for these differences.

More importantly, apart from enhancing the immune response to the tetanus toxoid vaccine, T3 was also found to promote tumor-induced immunity in 4T1 murine mammary carcinoma model. In mice injected with dendritic cells pulsed with 4T1 cancer cell lysate, daily oral supplementation of TRF was found to significantly suppress tumor onset after inoculation with 4T1 cells (31). This effect was associated with an enhanced production of the immune stimulatory cytokines interferon- γ and interleukin-12 by dendritic cells and splenocytes, suggesting that TRF induces cytokine release and promotes a tumor cell-mediated immune response (31).

Targeting cancer stem cells by T3

Cancer stem cells (CSCs), also known as tumor-initiating cells, are subpopulations of cells within cancers with self-renewal and multilineage potential and are believed to be responsible for the development of human tumors. CSCs were first identified in leukemia patients and later on in a wide range of solid tumors. They are characterized by their ability to self-renew, form spheroid structures *in vitro* and differentiating to form the bulk of the tumor *in vivo*. It is believed that early elimination of CSCs may confer cancer chemoprevention, although an anti-CSC-specific agent is yet to be developed. Interestingly, a recent study by our laboratory reported that the gamma form of T3 (γ -T3) has potent anti-CSC properties. We found that treatment of prostate cancer cell lines with γ -T3 significantly inhibited the spheroid formation ability of the cells, which was associated with the downregulation of CSC marker expression (32). Consistent with this, oral administration of γ -T3 was found to suppress tumor formation in >70% of mice subcutaneously injected with prostate cancer cells (32), further validating the anti-CSC effect of γ -T3. Intriguingly, γ -T3 treatment in prostate cancer cells was found to downregulate the protein levels of Id-1 and beta-catenin (32), two well-documented stem cell maintenance proteins that promote stem cell survival and self-renewal ability (33,34). However, the same treatment failed to affect the messenger RNA levels of either genes (unpublished data),

suggesting that γ -T3 may regulate the protein levels of Id-1 and beta-catenin through a post-transcriptional mechanism. Thus, although these findings clearly demonstrate the potential anti-CSC properties of γ -T3, the underlying mechanism is still far from clear.

Tocotrienols as an anticancer agent

Selective cancer cell-killing effect

In addition to its chemopreventive effect, ample evidence suggests that T3 may also be an effective cancer therapeutic agent. One intriguing finding, which was demonstrated consistently by us and others, is its selective killing effect against cancer cells. In our recent studies, T3 was found to induce apoptosis in prostate and breast cancer cells but not in the non-malignant breast and prostate epithelial cells (35,36), suggesting that it has selective cancer cell-killing properties. In addition, in both studies, the hormone-independent cell lines were found to be more sensitive to T3 than the hormone-dependent lines, which highlight the potential of T3 in targeting breast and prostate tumors at hormone refractory stage. Similar findings were reinforced by Srivastava *et al.* (37), wherein TRF was found to inhibit the growth and viability of prostate cancer cells; yet, the same treatment was unable to induce significant cell death in non-malignant prostate epithelial cells. This selectivity may in fact be due to the induction of interleukin-24 (IL-24) (38), a cytokine known to specifically induce apoptosis of cancer cells but not of the normal cells. A higher uptake of T3 by cancer cells may also account for the observed cancer cell-specific killing effect since examination of T3 distribution *in vivo* revealed that T3 tends to accumulate at a much higher concentration in tumor tissues when compared with other vital organs (39). Meanwhile, despite its low bioavailability in comparison with TP, T3 has a >16-folds higher uptake rate than TP, which may explain the ineffectiveness of TP as anticancer agent in our and other studies (32,40).

Therapeutic inhibition of cell migration and invasion

Liu *et al.* (41) demonstrated that in gastric adenocarcinoma SGC-7901 cells, γ -T3 treatment leads to inhibition of cell migration and matrigel invasion through downregulation of transcription of matrix metalloproteinases (MMP-2 and MMP-9) as well as upregulation of tissue inhibitors of metalloproteinase-1 (TIMP-1 and TIMP-2). Similarly, in two of our recent studies, γ -T3 was found to significantly inhibit the invasive ability of melanoma and prostate cancer cells (35,42). In addition, in both studies, γ -T3 was found to upregulate expression of epithelial markers, such as E-cadherin and gamma-catenin, whereas at the same time, downregulate the level of mesenchymal markers like vimentin and alpha-smooth muscle actin (35,42). These observations indicated that γ -T3 treatment inhibits cancer cell invasion through reversal of the epithelial-to-mesenchymal transition (EMT), a major mechanism associated with cancer metastasis and disease recurrence. It is also conceivable that the reversal of EMT by T3 may in part contribute to the anti-CSC properties of T3 discussed above because of the well-documented link between EMT and CSC.

Therapeutic inhibition of angiogenesis

The first study that demonstrated the antiangiogenic properties of T3 was performed by Inokuchi *et al.* (43), which revealed an inhibitory effect of T3 on proliferation and tube formation of bovine aortic endothelial cells. Similar results were reported recently by Shibata *et al.*, showing that the delta form of T3 (δ -T3) could completely abolish proliferation, migration and tube formation of human umbilical vein endothelial cells, whereas a similar dose of α -T3 had minimal effects (44). The vascular endothelial growth factor (VEGF) signaling pathway appears to be the major target of T3 because expression of both VEGF and VEGF receptor have been reported to be downregulated by T3 treatment (45). In gastric cancer cells, γ -T3 treatment was found to modulate the paracrine secretion of VEGF induced by cobalt (II) chloride via the extracellular signal-regulated kinase signaling pathway (45). Meanwhile, TRF supplementation was

found to significantly reduce serum VEGF levels in BALB/c mice (46). On the other hand, T3 treatment was also shown to downregulate VEGF receptor expression in endothelial cells (47). The concomitant decrease in both the ligand and the receptor of the VEGF signaling pathway may account for the severe inhibition of inflammation- or tumor-associated angiogenesis by T3 as reported in several recent studies (46,48,49).

Tocotrienols as adjuvant cancer therapy

Adjuvant therapies such as radiation or chemotherapeutic drugs are the treatments that are given in addition to surgery to suppress tumor relapse through elimination of any remaining cancer cells. These adjuvant therapies are designed to kill actively proliferating cancer cells but are often ineffective once the tumor metastasized. Furthermore, their effectiveness is often further hampered by the associated side effects and the development of treatment resistance. Recent studies (35,41–46,48,49) of T3 have provided some encouraging data demonstrating that it can, on one hand, inhibit tumor invasion and angiogenesis and, on the other hand, it can sensitize cancer cells to chemotherapy, suggesting that it can either be used alone or be used with additional chemotherapeutic agents as an effective adjuvant (Figure 2).

Dual effects of T3 as radioprotector for normal cells and radiosensitizer for cancer cells

Radiation therapy is one of the most widely used adjuvant anticancer therapies, which is often associated with adverse effects, with the hematopoietic system being the most vulnerable tissue to the treatment. Four recent reports have revealed the potential radioprotective nature of T3, particularly the gamma and delta isoforms. Li *et al.* (27) demonstrated that a single injection of δ -T3 protected 100% of CD2F1 mice from total-body γ -irradiation-induced death (8.75 Gy) 30 days post-irradiation compared with an 18% survival rate in the vehicle alone-injected group (27). Interestingly, δ -T3 increased cell survival rates, the regeneration of hematopoietic microfoci and Lin⁻Sca1⁺c-kit⁺ stem and progenitor cells in irradiated mouse bone marrow (27), suggesting that δ -T3 can promote hematopoiesis. The authors also demonstrated that δ -T3 protected human hematopoietic CD34⁺ cells from radiation-induced damage *in vitro* through the activation of Erk/mTOR signaling (45). On the other hand, Kulkarni *et al.* reported that γ -T3-treated mice exhibited a 90% recovery of Lin⁻c-kit⁺ hematopoietic stem cells seven days after total-body irradiation, whereas the vehicle-treated group remained depleted (26). Other studies have demonstrated that γ -T3 can accelerate the recovery of total white blood cells (50), ameliorate intestinal radiation injury and reduce vascular oxidative stress (51).

Intriguingly, T3 appears to protect hematopoietic stem cells, whereas at the same time, sensitize tumor cells towards irradiation-induced cell death. Kumar *et al.* studied the effects of γ -T3 on mice with prostate cancer xenografts after irradiation and found that subcutaneous injections of γ -T3 24 h prior to irradiation at the tumor site (5 Gy/min for a final dose of 12 Gy) resulted in a nearly 40% reduction of tumor size when compared with the vehicle-treated group (52). With a growing body of research suggests that CSCs are inherently resistant to radiation (53–56), it is conceivable that the anti-CSC properties of T3 may contribute to the radiosensitizing effect observed in their study. However, owing to the differences in radiation doses and exposure areas between the study by Kumar *et al.* (52) and by Kulkarni *et al.* (26), it remains unclear if the dual effects of γ -T3 occurred simultaneously. Thus, further investigation is necessary to understand the role of T3 in radiation therapy of cancer.

T3 as a potent chemosensitizer

T3 has been shown to sensitize cancer cells to different chemotherapeutic drugs. Reports from our laboratory suggested that in the presence of γ -T3, both melanoma and prostate cancer cells became highly sensitive to docetaxel-induced apoptosis, suggesting that γ -T3 sensitized these cells to docetaxel (35,42). Similarly, Kunnumakkara

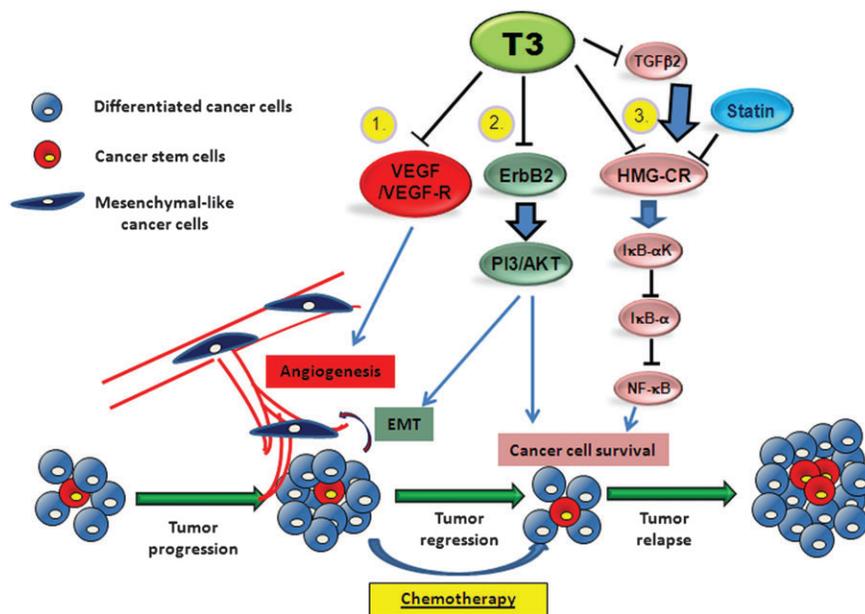


Fig. 2. Anticancer properties of Tocotrienol are associated with targeting of multiple signaling pathways. 1. Tocotrienol inhibits VEGF-dependent tumor angiogenesis through downregulating the expression of both VEGF and VEGF receptor. 2. It also inactivates the PI3/AKT signaling pathway by downregulating HER2/ErbB2 expression, which may associate with its suppressive effect on EMT. 3. Tocotrienol inhibits cancer cell survival through suppression of NF-kappa B pathway. Downregulation of the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase appears to play an important role in this process.

et al. (57) demonstrated that γ -T3 is capable of sensitizing pancreatic cancer to gemcitabine both *in vitro* and *in vivo*. In particular, oral administration of γ -T3 together with gemcitabine treatment was found to further reduce orthotopic pancreatic tumor volume when compared with the groups treated with either agent alone (57). Recently, using both breast and pancreatic tumor xenograft models, we have confirmed the chemosensitization effects of γ -T3. In mice implanted with breast cancer cells, docetaxel plus γ -T3 treatment was found to achieve much significant tumor-suppressive effect than either agent alone (Figure 3, Al-Ejeh *et al.*, unpublished data). Similarly, γ -T3 treatment was also found to sensitize pancreatic tumor to gemcitabine treatment (Figure 3, Al-Ejeh *et al.*, unpublished data), further validating the chemosensitizing effect of T3 on cancer cells.

In addition to classical chemotherapeutic drugs, T3 also showed chemosensitizing effects to long list of targeted cancer therapeutics. A combination of γ -T3 with atorvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, and celecoxib, a cyclooxygenase-2 inhibitor, acted synergistically to induce G₀/G₁ phase cell cycle arrest and apoptosis of HT29 and HCT116 colon cancer cells (58). In addition, γ -T3 was also reported to sensitize cancer cells to other targeted cancer therapeutics, such as the erlotinib (EGFR inhibitor), gefitinib (EGFR inhibitor), bortezomib (a proteasomal inhibitor), thalidomide (a TNF inhibitor) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (59–62) both *in vitro* and *in vivo*.

Signaling mechanisms underlying the anticancer effect of T3

Nuclear factor-kappaB

The anticancer effect of T3 has been linked with the inhibition of nuclear factor (NF)-kappaB signaling pathway (Figure 2). NF-kappaB is a key regulator in cell survival that regulates expression of a series of antiapoptotic proteins and is frequently upregulated in cancer cells. Previously, T3 but not TP has been shown to inhibit TNF- α -induced NF-kappaB activation in pancreatic cancer cells, leading to subsequent downregulation of the NF-kappaB-downstream targets that are associated with survival (IAP1, IAP2, Bcl-xL, Bcl-2, cFLIP, XIAP, Bfl-1/A1, TRAF1 and survivin), proliferation (cyclin D1, cyclooxygenase and c-Myc), invasion (matrix metalloproteinase-9 and ICAM-1) and angiogenesis VEGF (63). This inhibitory effect

of T3 is not restricted to pancreatic cancer cells as similar observation have been reported in skin (42) and prostate cancer cells (35) and also in normal monocytic cells (64). T3 appears to inhibit NF-kappaB pathway by stabilizing the NF-kappaB inhibitor I kappaB- α and suppressing the nuclear translocation of the NF-kappaB p65 subunit (63). Interestingly, earlier studies reported that T3 also downregulates TGF β 2 (65), which functions as an upstream regulator of the NF-kappa B pathway. Meanwhile, the inhibitory effect of T3 was reversed by addition of mevalonate, suggesting that T3 inhibits NF-kappaB through a 3-hydroxy-3-methylglutaryl coenzyme A reductase-dependent mechanism. However, unlike Statin, which functions as HMGCoR inhibitor, T3 treatment was found to downregulate the expression of HMGCoR protein.

PI3/AKT

The PI3/AKT signaling pathway plays an important role in the survival, proliferation and invasion of the cancer cells and is aberrantly activated in a wide range of cancers. As demonstrated by Shah *et al.*, in mouse mammary tumor cells that treated with γ -T3, a drastic reduction in the phosphorylation of Phosphoinositide-dependent Kinase-1 and AKT was observed (66), indicating a decrease in PI3/AKT activities. This inhibition was associated with suppression of the antiapoptotic protein FLICE-inhibitory protein and subsequent activation of caspase-8. More recently, both γ - and δ -T3 were found to downregulate the expression of Her2/ErbB2, an upstream activator of the PI3/AKT pathway and as a result inhibited the proliferation of pancreatic cancer cells (67). Considering the important role of the PI3/AKT pathway in CSC renewal and survival, the potent inhibitory effect of T3 on this pathway may also account for its recently revealed anti-CSC effect (Figure 2).

Current progress in the clinical development of T3-based cancer therapy

The accumulating evidence supporting the use of T3 as an adjuvant therapy has prompted researchers to study its effect in a clinical setting. A double-blind placebo-controlled pilot trial that recently completed tested the effectiveness of adjuvant tocotrienol therapy in combination with tamoxifen in women with early breast cancer

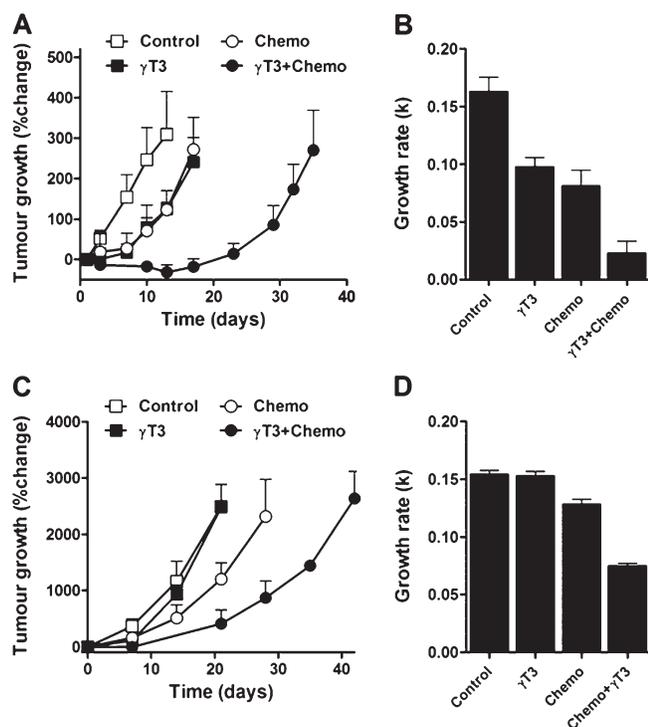


Fig. 3. Efficacy of γ -T3 treatment in combination with chemotherapy in breast and pancreatic cancer xenograft models. Female balb/c nude mice (6 weeks of age) were injected with human breast cancer cells (MDA-MB-231) in the mammary fatpad (A) or human pancreatic cancer cells (PANC-1) subcutaneously (C). The largest tumor diameter (a) and the smallest tumor diameter (b) were measured using callipers and tumor volume was calculated as per the equation: $b^2 \times a/2$. In (A), groups of five mice each were left untreated (control) or treated with a single intraperitoneal dose of 2 mg/kg of docetaxel on day 0 (chemo), five intraperitoneal doses of 50 mg/kg γ -T3 (in sunflower oil) administered daily on days 1, 2, 3, 4 and 5 (γ -T3) or the combination of docetaxel (day 0) and γ -T3 (days 1–5). In (C), groups of five mice each were left untreated (control) or treated with intravenous doses of 50 mg/kg gemcitabine on days 1, 4, 7 and 10 (chemo), five intraperitoneal doses of 50 mg/kg γ -T3 (in sunflower oil) administered daily on days 1, 2, 3, 4 and 5 (γ -T3) or the combination of gemcitabine (days 1, 4, 7 and 10) and γ -T3 (days 1–5). Tumor volume was measured on the specified days after treatments and was fitted to exponential growth curves using GraphPad Prism to generate the growth rates (k/day) for the MDA-MB-231 (B) and PANC-1 (D) xenografts.

(68). Two hundred and forty women with estrogen receptor-positive breast cancer were assigned to the intervention group (TRF plus tamoxifen) or the control group (placebo plus tamoxifen) (68). Examination of the 5 year breast cancer-specific survival and 5 year disease-free survival revealed that survival rates were only slightly higher in women from the intervention group when compared with the control group, and the difference was not statistically significant (68). These data suggested that TRF might not be an effective adjuvant therapy for breast cancer patients undergoing tamoxifen treatment. Considering that TRF still contains TP, which may interfere with the absorption and the anticancer effect of T3, additional trials are necessary to determine if pure T3 isomer can achieve better results compared with TRF. Meanwhile, since different T3 isomers have different elimination half-life (4.3 h versus 2.2 h for δ -T3 and γ -T3 respectively) (6), separate pilot clinical trials testing each of these isomers will be essential for determining the toxicity profile and effective dosage. Recently, a phase I clinical trial was initiated to study the effect of δ -T3 supplementation on patients with resectable pancreatic cancer, which is expected to be completed in July 2013 (69). This trial is the first to test the pharmacokinetic and toxicology of a single T3 isomer on human subjects. Recent release of the preliminary data from this trial revealed that a dosage of up to 800 mg/

day was well tolerated by the pancreatic cancer patients, with further escalation to 3200 mg/day being planned (70). Completion of this trial will thus be the first step in determining the safety and toxicology profile of a single T3 isomer and providing the reference dosage for future phase II trial.

There is also an increasing interest in improving the anticancer effect of T3 by either enhancing the delivery of T3 or by direct modification of its chemical structure. Ali *et al.* (71) has recently described the use of the nanolipid carrier to efficiently deliver simvastatin and TRF into mammary cancer cells and revealed the existence of a synergistic anticancer activity. In addition, entrapping T3 into a transferrin-bearing multilamellar vesicles was also shown to enhance the antitumor effect of T3 by up to 70-folds (72). On the other hand, two recent studies also described the design and synthesis of the redox-silent T3 analogues, which led to an improved inhibitory effect on the proliferation and invasion of breast cancer cells when compared with the natural form of T3 (73,74). Overall, these studies have yielded promising data, which may benefit the future development of T3-based anticancer therapy.

Conclusions

T3, especially the gamma and delta isoforms, exhibits superior anticancer effects in comparison with TP, which include anti-inflammation, anti-invasion, antiangiogenesis, chemosensitization (Figure 2) and radiosensitization. Recent efforts also suggest that T3 has potential anti-CSC properties, providing new hope for the elimination of chemo-resistant residual tumor cells, which have recently been suggested to be responsible for tumor recurrence and relapse after therapy. Growing interest in T3 will doubtlessly increase our mechanistic understanding of its unique anticancer properties, such as the targeting of CSCs or reversal of EMT. With the promising results observed in a number of recent pre-clinical studies, research focus should now be put on the design of additional clinical trials to test the anticancer effect of T3 (particularly the pure single isomer). Specifically, the potent effect of gamma-T3 on the hormone refractory breast and prostate cancer cells warrants further investigation of its therapeutic potential on patients with disease relapse after hormone ablation therapy. The chemosensitizing effect of T3 on systemic chemotherapeutic drugs such as docetaxel and gemcitabine should also be tested in clinical setting as that may yield considerable improvements in the management of advanced disease by overcoming therapeutic resistance.

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