Review Article

Tocotrienol and cancer metastasis

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Abstract

Tumor metastasis involves some of the most complex and dynamic processes in cancer, often leading to poor quality of life and inevitable death. The search for therapeutic compounds and treatment strategies to prevent and/or manage metastasis is the ultimate challenge to fight cancer. In the past two decades, research focus on vitamin E has had a shift from saturated tocopherols to unsaturated tocotrienols (T3). Despite sharing structural similarities with tocopherols, T3 strive to gain scientific prominence due to their anti-cancer effects. Recent studies have shed some light on the anti-metastatic properties of T3. In this review, the roles of T3 in each step of the metastatic process are discussed. During the invasion process, signaling pathways that regulate the extracellular matrix and tumor cell motility have been reported to be modulated by T3. Although studies on T3 and tumor cell migration are fairly limited, they were shown to play a vital role in the suppression of angiogenesis. Furthermore, the anti-inflammatory effect of T3 could be highly promising in the regulation of tumor microenvironment, which is crucial in supporting tumor growth in distant organs. © 2016 BioFactors, 42(2):149–162, 2016

Keywords: *cancer; metastasis; tocotrienols; invasion; migration; angiogenesis; anti-inflammation*

1. Introduction

Insights into treatment failure in cancer revealed that metastatic progression contributes to 90% of cancer-associated mortality. Metastasis is the end result of dissemination of tumor cells from the primary neoplasm to a distant organ and subsequent adaptation to foreign tissue microenvironment [1,2]. Data collected from Surveillance, Epidemiology, and End Results (SEER) program between 2008 and 2012 identified the top 15 highest cancer-related mortality rates categorized based on primary tumor sites in the United States (Fig. 1A). Patients with lung, colorectal and breast cancers are reported to be associated with highest death rates in all races. The development of metastatic disease often signals poor prognosis, giving rise to increased morbidity and mortality [1]. Figure 1B illustrates the stage distribution for cancers of solid tumors at diagnosis, classified by cancer histology into localized, regional (lymph node involvement), and distant (distant metastases) stages. Correlating with Fig. 1A, most cancers with high mortality rates are often associated with a high percentage of distant metastases, *i.e.* up to 50% in lung and bronchus cancer patients. Regional invasion accounted for up to 30% in colorectal, breast, and prostate cancers. Thus, the current focus of metastasis research aims to understand the entire metastatic process including micrometastasis and tumor microenvironment, rather than targeting the individual steps in therapeutic intervention [1].

Several chemoprevention and epidemiological studies have suggested that incidence of cancer can be well prevented by means of diet, lifestyle, and nutrition modification [3]. Over the past two decades, nutraceuticals, or plant derived dietary agents, have gained an important role in the quest to search for novel cancer therapeutics. One of the major advantages of nutraceuticals lies in their multitargeting properties, especially in cancer therapy. Despite the emergence of many new anticancer agents, effective drugs to eliminate metastasis and inevitable deaths in cancer remains a challenge. Multiple therapeutic strategies and personalized treatment regime, as opposed to

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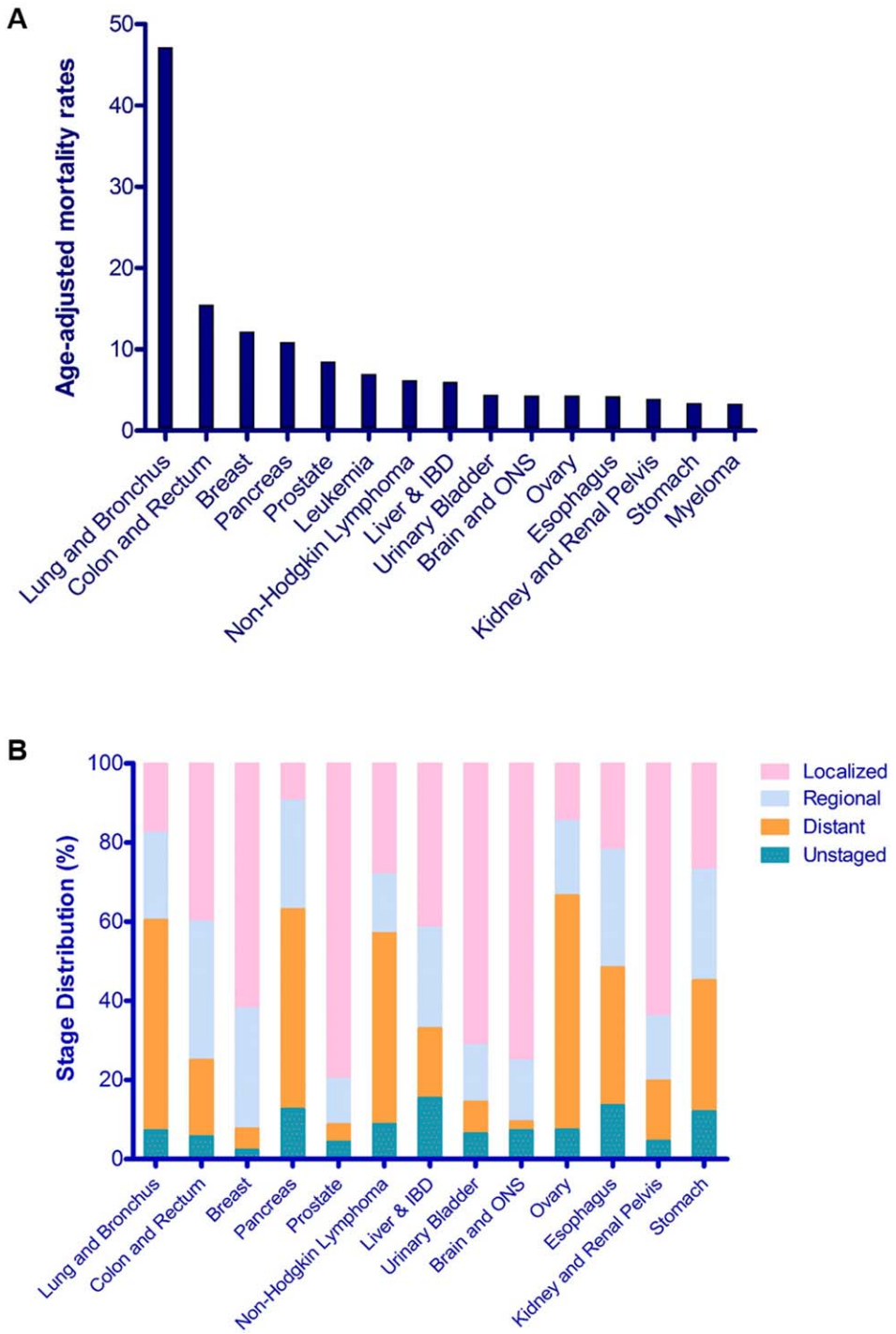
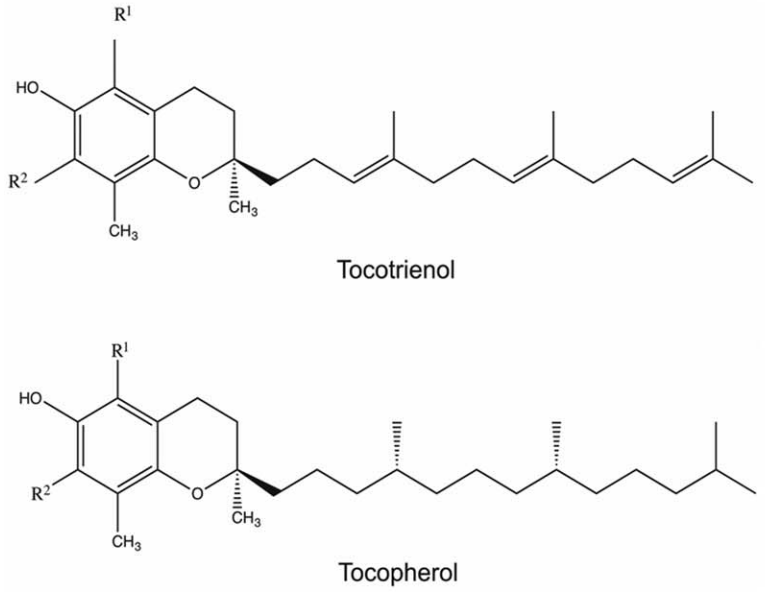


FIG 1

A. Surveillance, Epidemiology, and End Results (SEER) program top 15 highest cancer-related mortality rates based on primary tumor sites. Top 15 cancer sites were selected based on 2008 to 2012 age-adjusted rates for all races/ethnic group. B. Surveillance, Epidemiology, and End Results (SEER) program stage distribution of solid tumors at diagnosis. Incidence source were collected from 18 SEER areas in the United States. Stage distribution include only female data for breast and ovary cancers and only male data for prostate cancer. IBD = intrahepatic bile duct. ONS = other nervous system.

monotherapy, are in an increasing demand. Nutraceuticals such as curcumin, resveratrol, gambogic acid, capsaicin, and tocotrienol (T3) were shown to modulate the survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells [3]. In recent years, a growing body of evidence has suggested that T3 exhibits specific therapeutic functions in the regulation of tumor cells. T3 is a group of compounds from the vitamin E family, which are present in an assortment of components in the human



Tocotrienol	Tocopherol	R^1	R^2
α	α	CH_3	CH_3
β	β	CH_3	H
γ	γ	H	CH_3
δ	δ	H	H

FIG 2

Chemical structure of tocotrienol and tocopherol homologues.

diet. While tocopherol can be commonly found in vegetable fats and oils, T3 is readily available from palm oil, rice bran and annatto plant [4]. Consisting of the same aromatic chromanol "head," T3 has an unsaturated isoprenoid chain along its hydrocarbon tail, while tocopherol has a saturated phytyl side chain (Fig. 2). The four homologues of T3 and tocopherol, namely α, β, γ, and δ, are distinguished by the varying locations of their methyl groups on the aromatic ring.

The antiproliferative and proapoptotic effects of T3 *in vitro* and *in vivo* have been well documented. Numerous studies have proven the ability of T3 to suppress cancerous cell growth of breast, prostate, lung, bladder, liver, colorectal, and pancreas origin [5]. Significant tumor suppression in mice treated with T3, either as monotherapy [6] or in combination with chemotherapeutic agents [7,8] have been reported in animal models. In these studies, the proapoptotic properties of T3 was proposed as the major attribute to their anticancer effects. In clinical settings, a pilot trial using T3 as an adjuvant to tamoxifen in women with early breast cancer has been conducted [9]. Unfortunately, the 5-year breast cancer-specific survival and recurrence-free survival did not show statistical improvement in the group supplemented with T3. When analyzed using relative risk, breast cancer-specific death and recurrence were found to be 0.33 and 0.80, respectively [10]. Although lower risk of death and recurrence (70% and 20%, respectively) were found in the T3 group based on relative risk analysis, a notable weakness of this trial is the small sample size leading to low incidence of primary and secondary outcomes. Hence, a large randomized controlled trial is warranted to establish the efficacy of T3 as an adjuvant therapy in breast cancer.

The development of metastasis requires cancer cells to possess several abilities. Invasion itself is a multistep process, initiated by the adherence of tumor cells to the extracellular matrix (ECM). In most tumor cells, adhesions to ECM are often accompanied by degradation of the ECM at the other edge of the cell, thus stimulating cell motility and local invasion [1]. Following invasion, tumor cells have to enter the blood circulation and are required to survive in the blood circulation before extravasating to gain access into the metastatic site [2]. In circulatory system, transitions from epithelial to mesenchymal and vice versa are the major mediators for the survival of circulating tumor cells [11]. However, successful survival of tumor cells in the blood circulation does not necessarily correlate with the end of the metastasis cascade, *i.e.* colonization of secondary tumor sites. Colonization is a structured process dependent on the receptive microenvironment of distant tissues. The expansion of tumor vasculature is a major driver of successful tumor colonization at metastatic sites. Angiogenesis involves a chain of events leading to the establishment of new blood vessels, which provide oxygen and nutrient supplies to the tumor cells [12]. Chronic inflammation also plays a pivotal role in tumor microenvironment as it leads to a plethora of events including macrophage plasticity, genetic instability, and changes in immune functions [13]. Alterations of microenvironment by proinflammatory cytokines and chemokines, classic components of the senescence-associated secretory phenotype (SASP) may cause the microenvironment to accommodate tumor growth and progression. In an ideal situation, the SASP initiates the immune system to eradicate senescent cells. However, the SASP effectors together with changes in the genetic makeup may modify this protective tumor suppressive mechanism and cause the cancer cells to grow aggressively [14,15]. Therefore, the balance between engaging cellular senescence and the secretory profile of senescent cells in tumor

microenvironment may provide a therapeutic avenue in cancer treatment.

A growing abundance of studies are unveiling the potentially dynamic interactions between T3 and metastatic cancer cells. Table 1 reports a critical overview of major metastatic pathways modulated by T3 categorized according to types of T3 and cell lines. Years of efforts in cancer research have offered an enormous advancement in our understanding of metastasis. Here, the potential role of T3 and their individual homologues in the steps of metastasis, *i.e.* invasion, migration, angiogenesis, and modulation of inflammatory pathways will be discussed.

2. Effect of Tocotrienol on Invasion

The initial steps of local invasion involves alterations in tumor cell adherence to adjacent cells and to the ECM, proteolytic degradation of surrounding tissue and increase in intrinsic motility to physically propel tumor cells through the tissue [1,16]. Various studies have previously reported the anti-invasive effect of γ-T3 in cancer cells. For example, Yap et al. [17] investigated the invasive ability of palm γ-T3 to halt invasion on prostate cancer cells using a Matrigel invasion assay. The study showed that prostate cancer cells treated with 25 to 51 μM of γ-T3 for 24 h exhibited a 2.5-time suppression of invasive ability in comparison to the untreated control. The study further revealed that inhibition of cell invasion was not affected by cell growth, as the number of viable cells remained unchanged [17]. Similar results were observed in melanoma cells treated with 10 μM γ-T3 derived from palm. In a separate study using annatto δ-T3, a 2-fold suppression on cell invasion was observed at a concentration ranging from 20 to 30 μM in non-small cell lung carcinoma (NSCLC) [18,19]. Combinational treatment of cisplatin and δ-T3 has also been found to effectively reduce the invasive capability of NSCLC cells in comparison to either agent alone [20].

2.1. Cell Adhesion

Cell adhesion is part of the sequential events, which triggers the invasion of cancer cells. Suppression of tumor cell invasion into the basement membrane can be achieved through the prevention of cell adhesion [21]. *In vitro* studies with γ-T3 (12–60 μmol/L) reported a dose dependent inhibition of human gastric cancer cells attachment to fibronectin (FN) and laminin (LN), the principal constituents of the ECM [21]. Cadherins, which are responsible for the mediation of cell-cell adhesions, also play a role in cell invasion [1]. Intracellularly, cadherins send signals to catenins and the actin cytoskeletons, whereas the extracellular domains of cadherins bind cells through their homophilic protein-protein interactions [1]. Therefore, cadherins are important proteins for cell binding and the down-regulation of E-cadherin expression has been reported to be a common characteristic of metastatic cancers [1,17]. Up-regulation of E-cadherin has been reported in human malignant melanoma and prostate cancer cells treated with γ-T3 [17,19]. This is postulated to take place through proteins such as vinculin, which has been previously proven to play a role in establishing E-cadherin-based cell adhesion complex [17,19].

2.2. ECM and Surrounding Tissues

Solid tumors are commonly embedded within a complex network of ECM, basal membrane and vasculatures, which pose as physical barriers to metastatic tumor cells [2]. Proteolytic degradation of ECM is not only a prerequisite for tumor dissemination but also serves as an essential step in the invasive processes of cancer cells [2]. Matrix metalloproteinases (MMPs) are key proteases involved in the degradation of basement membrane and ECM components [2]. Specifically, MMP-9 has the ability to degrade gelatins, a major constituent of basement membrane therefore permitting the invasion of cancer cells into the blood or lymphatic vessels to travel and metastasise to other tissue organs [2]. Ahn et al. [22] was the first to report that γ-T3 effectively inhibited tumor necrosis factor (TNF)-induced MMP-9 expression, thereby inhibiting the degradation of ECM [22]. A study by Ji et al. [20] investigating the effects of cisplatin in combination with δ-T3 in NSCLC cells found that δ-T3 alone and in combination, suppressed the expression of MMP-9. *In vivo* studies using γ-T3 alone and in combination with gemcitabine further showed down-regulation of MMP-9 expression in pancreatic cancer cells [7]. In contrast, previous studies have reported that the overexpression of tissue inhibitors of metalloproteinases (TIMP)−1 and TIMP-2 play a role in the inhibition of tumor growth, invasion and metastasis [21,23]. A study by Liu et al. [21] found that at concentrations of 30, 45, and 60 μmol/L, γ-T3 significantly up-regulated the expressions of TIMP-1 and TIMP-2 in human gastric cancer cells. Collectively, these data suggest that T3 may be involved in the proteolytic degradation of ECM via the down-regulation of MMP-9 expression and up-regulation of TIMP-1 and TIMP-2 expression [21].

While cellular senescence is known as a tumor suppressive mechanism, recent studies reported that the altered secretory activities in senescent cells seemed to behave like a double-edged sword in tumorigenesis. The SASP, which consists of a variety of soluble and insoluble factors, can affect surrounding cells through its activation of different pathways that facilitate tumor progression and invasion [14]. As reported in Coppe et al. [14], MMP-1, -3, -10, -12, -13, and -14 are among the proteases up-regulated in SASP. In breast cancer, higher invasiveness and tumorigenicity were reported as a result of MMP-2 and MMP-3 secretion by senescent fibroblasts [24]. The expression of MMP-3 by senescent cells have also been linked to the disruption of mammary epithelial cells and inhibition of differentiation markers [14,24]. T3 was previously reported to potentially induce cellular senescence in selected cancer types. In breast cancer cells, γ-T3 and δ-T3 at concentrations of 50 and 100 μmol/L has been shown to induce the expression of senescent cell markers (p53, p21 and p16) [25,26]. This was further proven in a HER2/neu breast cancer mouse model where the administration of annatto T3 (90% δ-T3 and 10% γ-T3) not only resulted in delayed tumor onset but also a reduction in tumor

TABLE 1 *Cell lines/animal models and the metastatic pathways affected by tocotrienol*

Author (year)	*Type of tocotrienol*	*Cell line/Animal model*	*Findings*
Ye et al. (2015) [6]	δ-T3	Bladder cancer T24 and 5637 cells	↓ STAT3 activation ↑ tyrosine phosphatase SHP-1
Zhang et al. (2015) [27]	TRF	Colon cancer SW620 cells	↓ Wnt protein and β-catenin
Siveen et al. (2014) [29]	γ-T3	HUVEC and human liver carcinoma HCCLM3 cells	↓ phosphorylation of VEGFR-2 ↓ AKT/mTOR/P70S6 kinases activation
Nasr et al. (2014) [31]	α-T3, β-T3, γ-T3, δ-T3	Human hepatocellular carcinoma Hep G2 cells	↓ VEGF and VEGFR
Ayoub et al. (2013) [33]	γ-T3	Human mammary cancer MCF-7 and MDA-MB- 231 cells	↑ E-cadherin, β-catenin and CK18 ↓ vimentin
Husain et al. (2013a) [35]	δ-T3	LSL-Kras$^{G12D/+}$; Pdx-1-Cre pancreatic cancer mouse model	↓ NF-κB activation ↑ E-cadherin ↓ vimentin, VEGF, ICAM-1 and VCAM-1 ↑ CK18
Husain et al. (2013a) [35] (2013b) [36]	δ-T3	LSL-Kras$^{G12D/+}$; LSL-Trp53$^{R172H/-;}$ Pdx-1-Cre (KPC) pancreatic cancer transgenic mouse model	↑ CK18
Nakagawa (2013) [38]	α-T3, β-T3, γ-T3, δ-T3	Human colorectal adenocarcinoma DLD-1-CM cells	↓ VEGF, VEGFR-2 and HIF-1α ↓ phosphorylation of PI3K/PDK/Akt pathway proteins ↑ phosphorylation of ASK-1 and p38
Ji et al. (2012) [20]	δ-T3	Human NSCLC A549 and H1650 cells	↓ MMP-9, VEGF and NF-κB DNA binding activity ↑ caspase-3 and PARP
Manu et al. (2012) [8]	γ-T3	Human gastric cancer SNU-5 and SNU-16 cells	↓ NF-κB activation ↓COX-2, ICAM-1, CXCR4, VEGF, and MMP-9
Selvaduray et al. (2012) [40]	TRF, δ-T3	HUVEC and mouse mammary cancer 4T1 cells	↓ VEGF, VEGFR-1 and VEGFR-2 ↓ IL-8 and IL-6
Ji et al. (2011) [18]	δ-T3	Human NSCLC A549 and H1299 cells	↓ MMP-9, VEGF and NF-κB DNA-binding activity
Campbell et al. (2011) [42]	γ-T3	Human prostate adenocarcinoma PC-3 and LNCaP cells	↓ TGFβ receptor 1 and phosphorylated-SMAD-2 signaling
Ayoub et al. (2011) [44]	γ-T3	Neoplastic highly malignant +SA mammary epithelial cells	↓ Met activation

TABLE 1 *(Continued)*

Author (year)	*Type of tocotrienol*	*Cell line/Animal model*	*Findings*
Li et al. (2011) [45]	γ-T3	Human gastric adenocarcinoma SGC-7901 cell	↓ Wnt pathway signaling ↓ phosphorylation of VEGFR-2 ↓ β-catenin and MMP-9
Husain et al. (2011) [43]	α-T3, β-T3, γ-T3, δ-T3	Pancreatic ductal epithelial HPDE6-C7 and HPDE6 C7-KRas cells	↓ NF-κB activation
Rajendran et al. (2011) [34]	γ-T3	Human hepatoma HepG2, C3A, SNU-387 and PLC/PRF5 cells	↓ STAT3 activation and VEGF ↑ tyrosine phosphatase SHP-1
Pierpaoli et al. (2010) [25]	γ-T3, δ-T3	Human breast cancer SKBR3 and MCF-7 cells	↑ expression of p53, p21, and p16
Liu et al. (2010) [21]	γ-T3	Human gastric adenocarcinoma SGC-7901 cells	↓ MMP-2 and MMP-9 ↑ TIMP-1 and TIMP-2
Kunnumakkara et al. (2010) [7]	γ-T3	Human pancreatic cancer BxPC-3, MIA PaCa-2, and Panc-1 cells	↓ NF-κB activation ↓ MMP-9, ICAM-1, VEGF, and CXCR4
Selvaduray et al. (2010) [48]	TRF, α-T3, β-T3, γ-T3, δ-T3	Mouse mammary cancer 4T1 cells	↑ IL-8, IL-24 and VEGF
Kannappan et al. (2010) [49]	γ-T3	Human multiple myeloma U266, MM.1R, and MM.1S (dexamethasone-sensitive) cells and human pancreatic carcinoma MIA PaCa-2, human prostate cancer PC-3 and DU-145 cells	↓ STAT3 activation and VEGF ↑ tyrosine phosphatase SHP-1
Shirode and Sylvester (2010) [30]	γ-T3	Highly malignant +SA mouse mammary epithelial cell	↓ NF-κB activation ↓ PGE_2, COX-2
Chang et al. (2009) [19]	α-T3, β-T3, γ-T3, δ-T3	Melanoma G361 and C32 cells	↑ E-cadherin and γ-catenin ↓ twist, α-SMA, and vimentin
Shibata et al. (2009) [51]	δ-T3	HUVEC	↓ phosphorylation of PI3K/PDK/Akt pathway proteins ↓ phospho-PTEN ↓ phosphorylation of VEGFR-2 ↑ phosphorylation of ASK-1 and p38 ↑ caspase-3, −8 and −9
Yam et al. (2009) [52]	TRF, α-T3, β-T3, γ-T3, δ-T3	RAW264.7 macrophage	↓ IL-6 and NO ↓ PGE_2 and COX-2
Weng-Yew et al. (2009) [54]	TRF and δ-T3	Mouse mammary 4T1 cancer cells, BALB/c mice	↓ serum VEGF

TABLE 1 *(Continued)*

Author (year)	*Type of tocotrienol*	*Cell line/Animal model*	*Findings*
Miyazawa et al. (2009) [12]	α-T3, β-T3, γ-T3, δ-T3	HUVEC	↓ phosphorylation of PI3K/PDK/Akt pathway proteins ↓ phosphorylation of VEGFR-2 ↓ERK1/2, eNOS, and GSK3 α/β ↑ phosphorylation of ASK-1 and p38
Shibata et al. (2008b) [65]	δ-T3	HUVEC stimulated by human colorectal adenocarcinoma DLD-1 cells	↓ phosphorylation of VEGFR-2 ↓ phosphorylation of PI3K/PDK/Akt pathway proteins ↓ eNOS, GSK3 α/β and ERK1/2 ↑ phosphorylation of ASK-1 and p38
Shibata et al. (2008a) [4]	α-T3, β-T3, γ-T3, δ-T3	Human colorectal adenocarcinoma DLD-1 cells and human hepatoma HepG2 cells	↓ VEGF, IL-8, HIF-1α and COX-2 ↑ HIF-1α degradation
Wu et al. (2008) [55]	TRF	Human leukemia monocytic THP-1 cells	↓ NF-κB activation ↓ NO, iNOS, COX-2 and PGE_2
Yap et al. (2008) [17]	α-T3, β-T3, γ-T3, δ-T3	Human prostate adenocarcinoma PC-3, LNCap and PZ-HPV7cells	↑ E-cadherin and γ-catenin ↓ vimentin, a-SMA and twist
Nakagawa et al. (2007) [37]	α-T3, β-T3, γ-T3, δ-T3	BAEC and HUVEC	↓ phosphorylation of PI3K/PDK/Akt pathway proteins ↓ ERK1/2, eNOS, and GSK3 α/β ↓ phosphorylation of ASK-1 and p38
Ahn et al. (2007) [22]	γ-T3	Human myeloid KBM-5 cells, human lung adenocarcinoma H1299 cells, human embryonic kidney A293 cells, human breast cancer MCF-7, multiple myeloma U266 cells and human squamous cell carcinoma SCC-4 cells	↓ NF-κB activation ↓ MMP-9, ICAM-1 and VEGF
Sylvester et al. (2005) [41]	γ-T3	Highly malignant +SA mouse mammary epithelial cells	↓ phosphorylation of PI3K/PDK/Akt pathway proteins ↓ NF-κB activation
Miyazawa et al. (2004a,2004b) [56,57]	α-T3, β-T3, γ-T3, δ-T3	HUVEC	↓ VEGF and VEGFR
Miyazawa et al. (2004a,2004b) [56,57]			↓ NF-κB activation ↓ MMP-9, ICAM-1 and VEGF
Theriault et al. (2002) [39]	α-T3	HUVEC	↓ NF-κB activation ↓ ICAM-1 and VCAM-1

number and size through the enhancement of *in situ* apoptosis and senescence markers [32]. Nevertheless, the effect of T3 on SASP is still lacking in the scientific community and is certainly an area of great interest in cancer research.

2.3. Epithelial-Mesenchymal Transition (EMT)

Epithelial-mesenchymal transition (EMT) is one of the initiating steps involved in primary tumor invasion, a process imperative in the progression of cancer cells to the metastatic stage [17,19]. During EMT, tumor cells confer stem-like properties and a migratory phenotype through the loss of epithelial markers and increase in mesenchymal traits, which favours invasion [2,13]. EMT is also accountable for the mediation of single-cell invasion through the loss of intercellular adhesion and epithelial polarisation [13]. It has been proposed that the suppression of E-cadherin expression may be responsible for the promotion of EMT [17,19]. γ-T3 has been demonstrated to inhibit cell invasion through the up-regulation of E-cadherin and γ-catenin protein expression and down-regulation of mesenchymal markers (vimentin, α-SMA and twist) in human malignant melanoma [19] and prostate cancer cells [17]. A study by Campbell et al. [42] also reported that γ-T3 at 20 μM, down-regulated transforming growth factor-β (TGFβ)−2 and its downstream effects such as phosphorylated-SMAD-2 signaling in human prostate cancer. Disruption of SMAD signaling by γ-T3 was postulated to play a role in the prevention of EMT in prostate cancer [42].

Excessive Met signaling has been linked with aggressive malignant phenotypes [33]. Binding of hepatocyte growth factor (HGF) to Met stimulates cell motility and surrounding Met-expressing epithelial cells, thereby acting as a potent inducer of EMT in various cell types [33]. A recent study revealed that γ-T3 effectively down-regulated total Met levels with inhibition of HGF-dependent Met activation in highly malignant +SA mouse mammary epithelial cells [51]. Ayoub et al. [33] recognised that γ-T3 given in combination with a Met inhibitor up-regulated the expression of epithelial markers (E-cadherin, β-catenin, and cytokeratins 8/18) with a corresponding suppression in mesenchymal marker (vimentin) resulting in the blockade of EMT [33].

3. Effect of Tocotrienol on Cell Migration

Migration of cancer cells to a distant tissue or organ other than the primary tumor is an integral part of the metastasis process and the spread of cancer cells. In effect, the migration mechanisms of cancer cells are found to be similar to those that occur in normal, non-cancerous cells during physiological processes such as immune-cell trafficking, embryonic morphogenesis and wound healing [16]. Wound healing assay, also known as cell migration assay, is commonly used to study the extent of cancer cell migration *in vitro*. Typically, cancer cells grown to confluency will be "wounded" by scratching some cells off the monolayer, and the width of wound will be measured overtime to quantify the migration rate of the cells [28]. Compounds with anti-metastatic properties are expected to slow down the rate of migration of the cancer cells. Numerous studies have looked at T3's effect on cancer cell migration using this technique. Weng-Yew et al. [54] who conducted a cell migration assay on human umbilical vein endothelial cells (HUVEC) using the wound healing model, showed that tocotrienol-rich fraction (TRF) caused 49% and 53% reduction in the rate of wound healing at 8 μg/mL and 10 μg/mL, respectively. On the other hand, δ-T3 caused 48% and 76% reduction in the rate of wound healing at 8 μg/mL and 10 μg/mL, respectively [54]. The ability of δ-T3 in halting cell migration has also been observed in several cell migration assays. Shibata et al. [51] studied the effects of δ-T3 on HUVEC migration and showed that at 2 μM, δ-T3 suppressed VEGF-induced migration by 50%. Similarly, Ji et al. [18] proved dose-dependent inhibition of NSCLC cells migration after 20 h treatment of δ-T3 at 10, 20, and 30 μM. A study by Liu et al. [21] further supported γ-T3's ability to inhibit *in vitro* human gastric cancer cell migration. In VEGF-stimulated HUVEC migration assay, γ-T3 suppressed the migratory potential of the HUVEC after a 12 h treatment at a concentration of 50 μM [29]. In an *in vivo* study by Husain et al. [35], LSL-Kras$^{G12D/+}$; Pdx-1-Cre pancreatic cancer mouse models were treated with δ-T3. Vehicle-treated and non-treated mice both harbored invasive carcinoma of 10% and 8%, respectively; whereas δ-T3-treated mice demonstrated no signs of invasive cancer [35].

3.1. Chemokine Receptors

Members of the chemo-attractant cytokines (commonly known as chemokines) and their receptors have been shown to play crucial roles in several steps in tumorigenesis and/or metastasis [61]. C-X-C chemokine receptor type 4 (CXCR4) is the most common chemokine receptor shown to be overexpressed in over 23 different human malignancies including breast cancer, ovarian cancer, melanoma and prostate cancer [59]. Kunnumakkara et al. [7] testified that the usage of γ-T3, alone and in combination with gemcitabine, down-regulated the expression of CXCR4 in a dose-dependent manner in human pancreatic MIA PaCa-2 cells. When examining the pancreatic tumor tissues harvested from orthotopically implanted nude mouse model of MIA PaCa-2, the combination dose of 10 μmol/L γ-T3 and 200 nmol/L of gemcitabine was proven to inhibit the expression of CXCR4 genes to a greater extent than the individual treatment of either agent [7]. In a study using human gastric cancer cell line, Manu et al. [8] demonstrated suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-regulated expression of CXCR4 by γ-T3 in a time-dependent manner. Western blot analysis validated the declining level of CXCR4 over 24 h after being treated with 50 μmol/L of γ-T3. The authors also showed that γ-T3 potentiated the anticancer effects of capecitabine in gastric cancer cells [8]. The results from the study jointly point towards γ-T3's capacity in inhibiting the expression of genes involved in metastasis of gastric cancer cells.

3.2. Circulating Tumor Cells

During migration, cancer cells that are shed into the circulation and disseminate between the primary tumor and the metastatic site are known as circulating tumor cells (CTCs). As a matter of fact, CTC numbers in metastatic cancer patients are correlated with enhanced cell migration and invasion [62]. Furthermore, the relationship between CTC numbers and the extent of cancer has also been proven in patients with metastatic breast [63], lung [64], colon [62], and prostate [65] cancers. Several studies have looked at the effect of T3 treatment on CTCs. As limited studies have reported the direct measurement of T3's effect on CTCs, cytokeratin (CK 18) was used as a surrogate marker of circulating apoptotic epithelial cells [35]. This is based on the fact that CK 18 fragments are released into the extracellular space as a result of caspase digestion during the intermediate stage of apoptosis [53]. In LSL-Kras$^{G12D/+}$; Pdx-1-Cre pancreatic cancer mouse models treated with δ-T3, the results revealed a significant increase in the plasma levels of CK 18 in mice treated with δ-T3 [35]. The authors then concluded that δ-T3 is able to induce apoptosis of the CTCs with an oral intake of 400 mg/kg/day [35]. When used in combination with gemcitabine, δ-T3 was again shown to induce circulating cancer cell death by raising the levels of CK 18 in LSL-Kras$^{G12D/+}$; LSL-Trp53$^{R172H/-}$; Pdx-1-Cre (KPC) transgenic mouse model of pancreatic cancer [35]. These serve as evidence of δ-T3's ability in halting the migration of cancer cells by inducing apoptosis of the CTCs.

4. Effect of Tocotrienol on Angiogenesis

Angiogenesis is the formation of new blood vessels from preexisting vasculature, which is necessary for the transport of blood-borne nutrients to the tumor microenvironment for its growth and survival [12]. In the context of tumorigenesis, this phenomenon is triggered by a "switch" that tips the balance between the regulation of inducers and inhibitors [40,54]. T3 has justified its ability in inhibiting the process of angiogenesis in numerous preclinical studies. For example, a study by Shibata et al. [65] showed significant suppression of hemoglobin (Hb) content in the Matrigel plug assay when treated with δ-T3. *In vivo* evaluation of T3 on tumor angiogenesis revealed consistent observations [65]. In mice implanted with human colorectal adenocarcinoma cells, marked suppression of vessel formation was reported [38,51]. In a recent study, Siveen et al. [29] reported that γ-T3 inhibited vascular formation with a dose of 20 μg, showing almost no vasculature formation in an *in vivo* matrigel plug assay. γ-T3 was also found to inhibit microvessel formation *ex vivo* in rat thoracic aortic ring assay [29].

As an intricate process, angiogenesis involves the proliferation and migration of endothelial cells [54]. In HUVEC cells, Weng-Yew et al. [54] reported dose-dependent suppression of HUVEC proliferation of 16.1 to 73.6% when treated with TRF concentrations between 4 and 12 μg/mL. δ-T3 was found to be a more potent inhibitor of cell proliferation than TRF with $>$50% reduction in HUVEC proliferation from concentrations of 4 μg/mL onwards. These findings were consistent with other published studies which investigated the effect of T3 on VEGF and FGF-induced angiogenic processes in HUVEC and reported that all T3 isomers inhibited the proliferation of HUVEC in the order of potency of $\delta->\beta->\gamma->\alpha$-T3 [37,38]. The greater inhibition of proliferation by δ-T3 may be explained by its better incorporation into HUVEC [37].

In tubular morphogenesis, the effect of γ-T3 on HUVEC induced by gastric cancer cells was examined [44]. The study found suppressed tube formation after treatment with γ-T3 (10–40 μmol/L) without any changes in the luminal structure of the capillary tubes [44]. From the results, γ-T3 was suggested to be non-cytotoxic to endothelial cells as it inhibited capillary tube organization without affecting the existing capillary tubes [44]. Similar observations were reported when the effect of δ-T3 was investigated on colon cancer-induced tube formation [35]. δ-T3 was shown to suppress colon cancer-induced tube formation without affecting the luminal structure. When plotted at concentrations of 1 to 30 μM through a tube formation assay, all T3 isomers were found to reduce the width and length of bovine aortic endothelial cells (BAEC) endothelial tubes at a potency in the order of $\delta->\beta->\gamma->\alpha$-T3 [56]. It was also reported that lower concentrations of T3 were required for the inhibition of migration and tube formation in comparison to the concentration required for attenuation of cell proliferation [38].

4.1. VEGF and VEGFR-2

Angiogenesis also involves the release of specific molecules such as VEGF, FGF, and epidermal growth factor (EGF) by the tumor cells. These molecules are responsible for the activation of endothelial angiogenic gene expression as well as enhancing vascular permeability [35]. In patients with breast cancer, elevated expressions of VEGF has been correlated with increased intratumoral microvessel density with poor prognosis [54]. Tumors not only produce VEGF but may also induce production of VEGF from surrounding stromal tissues in order to stimulate angiogenesis [54]. Weng-Yew et al. [54] demonstrated that mice treated with 1 mg/day TRF for 5 weeks significantly suppressed serum VEGF levels by 51.5% in comparison to untreated mice. In the same study, serum VEGF was associated with a proportional correlation to tumor size [54]. Similarly, a study by Selvaduray et al. [48] ascertained that TRF and δ-T3 exert their antiangiogenic effects through down-regulation of VEGF expression in tumor cells.

Another key receptor in VEGF signaling is VEGF receptor (VEGFR)−2, which undergoes phosphorylation upon ligand binding and is fundamental for endothelial cell VEGF-stimulated proliferation, chemotaxis and survival [35]. VEGF binding and phosphorylation of VEGFR-2 have been shown to induce migration and tube formation in HUVEC [54]. δ-T3 and γ-T3 have been reported to suppress the phosphorylation of VEGFR-2 at Try1175 residue in a time dependent manner [28,65]. In a study by Miyazawa et al. [56], δ-T3 displayed potent anti-angiogenic effects through the suppression of VEGFR expression in HUVEC and inhibition of

intracellular VEGF signaling (phospholipase C and protein kinase C). *In vivo* studies by Selvaduray et al. [40] using BALB/c mice treated with TRF indicated significant reduction in RNA levels of VEGF, VEGFR-1, and VEGFR-2 in comparison to untreated group. They hypothesized that the suppressed binding of VEGF to VEGFRs is due to the reduced levels of VEGF in the circulatory system as well as reduced VEGFR-2 expression. Their results addressed the presence of smaller tumors in TRF-treated mice, suggesting a proportional correlation with VEGF levels [40]. T3, whether in independent administration or co-administration with epirubicin-loaded nanoparticles in HCC-bearing mice resulted in severe decline in hepatic VEGF level [31]. The study concluded that T3's inhibition of tumor-associated angiogenesis is VEGF-dependent, which occurs through the down-regulation of both VEGF and VEGF receptor expression [31].

Previous reports have shown that the suppression of Wnt signaling down-regulates mRNA and protein expression of VEGFR-2 [45]. In addition to its role in the process of cellular proliferation, differentiation, motility and morphogenesis, Wnt signaling also cross-talks with other signaling pathways responsible for the regulation of angiogenesis, invasion and metastasis [45]. The central player in Wnt signaling is β-catenin and γ-T3 was found to suppress the expression of total β-catenin and nuclear β-catenin in a dose-dependent manner [45]. A recent *in vivo* study by Zhang et al. [27] reported that TRF significantly decreased the expression of Wnt protein and β-catenin in human colon cancer xenografts in Balb/c nude mice. It was postulated that TRF and γ-T3 inhibitory actions on Wnt signaling might be responsible for the down-regulation of phospho-VEGFR-2 level, which in turn suppresses angiogenesis [27].

Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that is important in the mediation of angiogenesis in response to hypoxia as part of an adaptive response [5,35,38]. The binding of HIF-1α to hypoxia response element (HRE) is responsible for the activation of VEGF gene expression [38]. HIF-1α has been a molecular target for anti-cancer agents as raised levels of HIF-1α expression has been reported in various human cancers [38]. All four T3 isomers were shown to inhibit hypoxia-induced VEGF secretion in a dose dependent manner in both colorectal adenocarcinoma and liver hepatocellular carcinoma cells [4]. The study further revealed suppressive effects of δ-T3 on hypoxia-induced HIF-1α protein accumulation and HIF-1α–dependent transcriptional activity. Likewise, a study by Nakagawa [38] reported suppression of hypoxia-induced VEGF mRNA expression in human colon cancer cell line after treatment with δ-T3 as a result of the inhibition of hypoxia-induced HIF-1α protein accumulation.

4.2. PI3K/PDK/Akt Pathway

The activation of phosphoinositide 3-kinase/phosphoinositide-dependent kinase 1/protein kinase B (PI3K/PDK/Akt) signaling through the binding of VEGF to its receptors promotes cancer cell survival and represses apoptosis mechanisms [40,67]. In various cancer cell lines, T3 effectively inhibited the phosphorylation of PI3K/PDK/Akt pathway proteins [4,12,35,37,38,51]. Studies by Shibata et al. [35,51] showed that treatment with 5 μM of δ-T3 suppressed the intracellular levels of activated Akt in HUVEC stimulated by VEGF and human colorectal adenocarcinoma cells. On the other hand, tumor suppressor phosphatase and tensin homologue (PTEN) is a tumor suppressor that negatively regulates PI3K function and its downstream signaling events. PTEN has been reported to be frequently deleted or mutated in a variety of tumors, *i.e.* up to 70% in glioblastoma and 30% in advanced prostate cancer [67]. The loss of PTEN results in the activation of the PI3K/Akt signaling pathway and consequent tumorigenesis; as well as triggering senescence in mouse and human cells [26,51]. While studies by Shibata et al. [51] reported suppression of phospho-PTEN after treatment with δ-T3, a study by Sylvester et al. [41] on +SA mammary epithelial cells reported that treatment with 4 μM γ-T3 did not affect the total and phospho-PTEN levels despite significant suppression in phospho-Akt. Therefore, more studies into the PI3K/PDK/Akt pathway are certainly needed to elucidate if the effect of T3 is specific to different T3 isomers or cancer types that exhibit PTEN mutation.

In VEGF-induced HCC endothelial cells, γ-T3 was shown to suppress the mTOR signaling cascade and its downstream protein p70S6 kinases [29]. Integrating with growth factors, mTOR coordinates cell growth and proliferation. Inhibition of the mTOR pathway with drugs such as rapamycin has been frequently adapted as treatment strategy in cancer in order to promote apoptosis [26]. Yet, activation of mTOR is critically needed in the induction of cellular senescence through conversion of quiescent cells into senescent cells. It was evident from human and animal studies that cellular senescence is a crucial process for cancer suppression [26]. Contrary to the apoptosis pathway, some prosenescence anticancer strategies have been developed via hyperactivation of the mTOR pathway [68]. While T3 was reported to modulate cellular senescence and mTOR at multiple levels [15], mechanistic studies into this area are strongly needed to establish the missing link. Several studies have also reported the ability of δ-T3 to inhibit activation of signals downstream to PI3K/PDK/Akt such as endothelial nitric oxide synthase (eNOS), glycogen synthase kinase 3 (GSK3) α/β and extracellular-signal-regulated kinase (ERK) 1/2 to basal (non-stimulated) levels [12,37,65]. In addition, significant increase in caspase-3, -8, and -9 activities in VEGF-induced HUVEC treated with δ-T3 were found by Shibata et al. [51], indicating the role of caspase-dependent mechanisms in endothelial cell apoptosis.

4.3. Interleukins

Interleukins (IL)-6 and IL-8 are angiogenic cytokines that promote angiogenesis through the stimulation of platelets to increase VEGF levels [40]. IL-8 is also involved in the process of basement membrane degradation of endothelial cells during the formation of blood vessels through the production of MMPs [40]. An increase in IL-8 and its receptors has been correlated with increased proliferation and microvessel density in

prostate cancer [48]. In human colon cancer cells, δ-T3 effectively inhibited the secretion of IL-8 in hypoxic conditions via HIF-1α related pathways [4]. A study by Selvaduray et al. [40] exhibited a notable reduction in mRNA expression of IL-6 and IL-8 in TRF-treated HUVEC, with δ-T3 being the most potent isomer followed by γ-T3 [40]. The results were consistent with an earlier study that outlined a decrease in IL-8 expression in murine mammary carcinoma cells treated with TRF and δ-T3 [48]. The suppressed level of IL-6 in the presence of TRF has been suggested to contribute to the suppression of VEGF while the decreased levels of IL-8 prevents proliferation and migration of cells [40]. On the contrary, a dose dependent increase in IL-24 transcript expression was observed when murine mammary carcinoma cells were treated with 2 to 8 mg/mL of TRF and δ-T3 [48]. The same study also reported a two-fold increase in IL-24 mRNA levels in tumor tissues of BALB/c mice supplemented with TRF in comparison with control mice [48]. IL-24 is a tumor suppressing protein and has been reported to inhibit growth and metastasis in lung tumor [48]. Therefore, T3 modulates angiogenesis by lowering the transcription of pro-angiogenic factors (IL-8, IL-6 and VEGF) while elevating anti-angiogenic protein (IL-24).

4.4. HMG-CoA Pathway

The role of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors such as statins in modulating the angiogenic processes has been established in previous studies [5,69]. A study by Vincent et al. [69] reported that HMG-CoA reductase inhibitors suppress angiogenesis through the inhibition of intermediates farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP) in the HMG-CoA reductase pathway. Being a known inhibitor of HMG-CoA reductase [60], T3 was reported to alter the angiogenic mechanisms via the HMG-CoA pathway through the suppression of geranylgeranylation and membrane localization of a small GTP-binding protein, Ras homolog gene family, member A (RhoA) [45,46]. However, a study by Shibata et al. [65] revealed that FPP and GGPP did not abrogate the anti-tube formation property of δ-T3. Therefore, more studies to elucidate the mechanistic function of T3 on the HMG-CoA pathway are warranted.

5. Effect of Tocotrienol on Inflammation

Rudolf Virchow first hypothesized the relationship between cancer and inflammation in 1863. He postulated that cancer originated from sites of chronic inflammation and that the inflammatory event served as the cofactor to carcinogenesis [52]. The distinct properties of cancer cells, which allow them to undergo proliferation, metastasis, evasion of apoptotic signals and development of chemoresistance has been associated with the inflammatory response [47]. In fact, inflammation has been identified as the seventh hallmark of cancer [58]. The presence of proinflammatory cytokines, growth factors and angiogenesis indicate the relation between inflammation in the tumor milieu and disease advancement. Therefore, targeting the components of tumor microenvironment, especially inflammation, may be a challenging yet promising avenue in the battle against metastasis and recurrence of the disease [11]. T3 has been shown to play a role in the modulation of inflammation. It has been reported that γ-T3 has the potential to inhibit the activation of Nf-κB induced by TNF, phorbol myristate acetate, okadaic acid, lipopolysaccharide, cigarette smoke, IL-1, EGF and capecitabine [22,50]. Signal transducer and activator of transcription 3 (STAT3) pathway and cyclooxygenase-2 (COX-2) involved in inflammation and tumorigenesis have also been reported to be modulated by both γ-T3 and δ-T3.

5.1. Nuclear Factor Kappa B (Nf-κB)

Nf-κB is a family of transcription factors that regulate various proinflammatory genes linked with inflammation and cancer. Nf-κB and its regulated gene products have been linked with angiogenesis along with promotion of migration and invasion [7]. Previous studies have reported that γ-T3 suppressed both constitutive and inducible Nf-κB activation in human neoplastic mammary epithelial cells and HUVEC lines, which correlated with the suppression Nf-κB-regulated gene products such as COX-2, MMPs, intercellular adhesion molecule 1 (ICAM-1) and VEGF [7,22,39,50]. Gene products of Nf-κB involved in invasion such as COX-2, ICAM-1 and MMP-9 have also been reported to have an Nf-κB binding site in their promoter regions [50]. Ji et al. [18] evaluated the effect of δ-T3 on Nf-κB DNA-binding activity in NSCLC cells. The study found that δ-T3 notably suppressed Nf-κB DNA-binding activity and expressions of its downstream target genes [18].

Nf-κB also plays a major role in migration and invasion of pancreatic cancer cells and development of resistance to gemcitabine, thus increasing the mortality rate in pancreatic cancer. Husain et al. [43] showed that δ-T3 augmented the efficacy of gemcitabine activity in severe combined immunodeficiency (SCID) nude mice [43]. These findings were comparable with the observations of Kunnumakkara et al. [7]. Furthermore, δ-T3 given in combination with cisplatin was shown to cause significant suppression of cell growth, migration invasiveness, and induction of apopstosis in NSCLC cells in a dose dependent manner in comparison to either agents alone due to the inhibition of Nf-κB signaling pathways [20]. The combination treatment of δ-T3 and cisplatin proved greater effectiveness than either therapy alone due to cisplatin's DNA-damaging effect, which causes an increase in Nf-κB expression [20]. Whereas, δ-T3 counters the increase in Nf-κB expression through the suppression of NF-κB DNA binding activity in NSCLC cells thereby reducing drug resistance [20]. γ-T3 in combination with capecitabine also presented an advantage in suppressing the overexpression of Nf-κB-regulated gene products in tumor tissues [8].

5.2. Signal Transducer and Activator of Transcription 3

Constitutive activation of transcription factor signal transducer and activator of transcription 3 (STAT3), has been linked with the expression of gene products involved in several steps of the metastatic cascade with a particularly high correlation to

inflammation and tumorigenesis [49]. A recent study by Ye et al. [6] reported that δ-T3 suppressed the phosphorylation level of STAT3 in bladder cancer cells with increased expression of SHP-1, a negative regulator of STAT3. Another study that examined the modulation of STAT3 signaling pathway discovered that γ-T3 suppressed both constitutive and IL-6-induced STAT3 activation through induction of protein-tyrosine phosphatase SHP-1 [49]. Similar observations were made in human HCC cells in conjunction with inhibition of gene products downstream of STAT3 after treatment with γ-T3 [34]. Furthermore, down-regulation of VEGF as a result of STAT suppression was observed in cells treated with γ-T3 in a time-dependent manner [34].

5.3. Cyclooxygenase (COX)

Prostaglandin synthase family of enzymes is composed of constitutively expressed COX-1 enzymes involved in cellular 'housekeeping' functions and the inducible COX-2 enzyme that plays a crucial role in inflammation [47]. Overexpression of COX-2 and prostaglandin, a product from catalyzed oxidation of arachidonic acid, leads to various pathophysiological condition diseases such as cancer with increased angiogenesis, metastasis and anti-apoptosis. A cell culture study performed by Nakagawa [38] discovered that part of T3's anti-angiogenic effects is mediated via the reduction of COX-2. Wu et al. [55] reported a selective suppression of COX-2 but not COX-1 expression with reduction in PGE_2 in cells treated with T3. γ-T3 also demonstrated potent growth inhibition on cancer cells with limited or absence of toxicity to normal cells indicating the selective anti-cancer activity of γ-T3 [7,55]. A study by Shibata et al. [4] found that δ-T3 slightly reduced COX-2 protein levels but not the level of mRNA expression in human colon adenocarcinoma cells incubated in hypoxic conditions. In highly malignant mouse +SA mammary epithelial cells, a sub-effective dose of γ-T3 at 0.25 μM in combination with 2.5 μM of celecoxib, a COX-2 selective inhibitor, prominently decreased COX-2 expression and subsequent PGE_2 synthesis [30]. On the other hand, high doses of γ-T3 (3 μM) or celecoxib (20 μM) alone only showed a slight reduction in COX-2 levels. These findings validates that T3 exhibits synergistic activity at lower doses by potentiating the activity of celecoxib in lowering COX-2 levels.

6. Conclusion

It is evident from the *in vitro* and animal studies that T3 is profoundly engaged in the disruption of metastatic process. Observations from Matrigel assays suggested suppression of invasion and migration in cancer cells treated with T3 while animal models of pancreatic and mammary tumors indicated reduced invasion and number of secondary tumors in distant organs. Collective data from angiogenic studies suggested notable suppression of proliferation and tube formation in endothelial cells, in addition to reduced number of growing blood vessels in embryogenic model. Moreover, ample evidence from cell-based studies provides considerable mechanistic insights into the anti-metastatic effect of T3. When summarized in Table 1, it was evident that T3 exhibits multi-targeted functions at different stages of metastasis. We also note that the regulation of several major pathways including down-regulation of Nf-κB, VEGF, and MMP-9 were relatively consistent across the various cell types. With the aid of whole genome transcriptome analysis, modulation of novel pathways that were often unnoticed can now be identified at the gene level. For example, a meta-analysis of gene expression data found up-regulation of up to 8 genes that were involved in endoplasmic reticulum stress in T3-treated MCF-7 breast cancer (GEO DataSet GSE 21946) and HeLa cervical cancer cells (GEO DataSet GSE48668) [26,70]. Microarray results from these studies also point towards functional manipulations of cellular senescence in T3-treated cells. The role of senescence in metastasis is attracting considerable interest in cancer research. Interestingly, T3 is one of the dietary compounds reported to modulate cellular senescence at both the morphological and genetical level [26]. More studies in these areas are certainly required in order to unfold the mechanistic identity of T3 in cancer.

Nevertheless, clinical translation regarding the use of T3 as an anti-metastatic agent is not viable at this stage, as some critical questions remain unanswered. The number of animal studies evaluating T3 in the context of metastasis was very limited, with only 11 studies being identified in this review. The lack of *in vivo* data is complicated by several uncertainties in the bioavailability of T3. While it has yet to be clarified if T3 is adequately distributed to tumor tissues, the effect of dose, dosing schedule and dosage forms might show different levels of potency in their anti-metastatic efficacies. In view of the knowledge gap, more animal studies to investigate the correlation between bioavailability and efficacies with the aid of advanced imaging techniques are in high demand. Non-invasive imaging facilities equipped with tracers of very high sensitivity will provide real-time information on the distribution of T3 as well as the progression of metastasis. We could not stress enough that metastasis is a dynamic and complex process and that different treatment strategies are needed to establish multiple lines of defense against metastasis. Hence, integration of rapidly evolving knowledge in fields such as stem cell research, genomics and immunology may lead to a future where fatal metastatic diseases can be a manageable condition.

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