

APOPTOSIS GENE NETWORK REGULATED BY DELTA-TOCOTRIENOL IN K562 CHRONIC MYELOID LEUKAEMIA CELLS

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ABSTRACT

Palm oil is rich in various health-beneficial phytonutrients. The vitamin E and the carotenoids make up a large portion of these phytonutrients. The tocotrienols are a relatively newer class of vitamin E isoforms that are actively being researched for its various health promoting properties. In this study, the anti-cancer properties of the delta-tocotrienol (δ -T3) isoform were investigated on a leukemic cell line. A preliminary cytotoxicity assay was carried out to identify δ -T3's potency in inducing cell death. Following this a real time-based gene array experiment was carried out to distinguish the apoptosis genes regulated by δ -T3 in the K562 leukemic cell line. Results show that treatment with δ -T3 significant regulated the expression of several genes that promote apoptosis such as TP53, TP73, HRK, MCL1, CASP7, CASP8, DAPK1, PYCARD, FASLG and TNFRSF9. These findings suggest that palm δ -T3 can exert anti-cancer activities in chronic myeloid leukemic cells

Keywords: tocotrienol, apoptosis, cytotoxicity.

Date received: 12 May 2015; **Sent for revision:** 28 December 2015; **Received in final form:** 28 February 2017; **Accepted:** 26 April 2017.

INTRODUCTION

The oil palm is aptly known as the 'golden crop' due to its various beneficial uses. Palm oil which is extracted from the fruit of the oil palm contains various phytonutrients, especially vitamin E and the carotenoids (Bonnie and Choo, 2000). Vitamin E is a naturally-occurring antioxidant, which is made up of two main sub-families, namely tocopherols and tocotrienols (T3). Each sub-family can be further classified in four isoforms: alpha (α), beta (β), delta (δ) and gamma (γ) based on their chemical structures. The tocotrienols are relatively more

potent in inhibiting cancer cell growth compared to the tocopherols (Pierpaoli *et al.*, 2010a). Research has shown that tocotrienols possess potent anti-cancer activity such as anti-metastatic (Liu *et al.*, 2010), inhibition of lipid peroxidation and nitric oxides (Kamat *et al.*, 1997), anti-angiogenic (Weng-Yew *et al.*, 2009), pro-apoptosis (Loganathan *et al.*, 2013) and enhance cell-mediated immune responses (Nesaretnam *et al.*, 2004).

Cancer is a genetic disease, caused by malfunctions in various oncogenes, tumour suppressor genes and stability genes (Vogelstein and Kinzler, 2004). Mutations in oncogenes cause these genes to be constitutively active as opposed to their wild-type counterparts. Therefore, dysfunction of these genes can provide opportunities for carcinogenesis. Due to a cell's inherent protection, it has been argued that cancer cannot be due to a single gene defect and that that cancer occurs due to the accumulation of multiple defects in our genes (Vogelstein and Kinzler, 2004).

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It has been suggested that genes can cause disruptions in various physiological processes such as apoptosis or programmed cell death that can be used to eliminate 'rogue' or cancer cells (Lowe and Lin, 2000). Hence, it is becoming important to identify genes that play a role in allowing cancers to develop and evade the immune system so that we can understand the mechanisms that support or promote oncogenesis. Apoptosis is a crucial process to maintain normal tissue development and function. Previously, the chemotherapeutic drugs used were designed to target accelerated cell growth, based on the premise that tumour cells had a faster growth rate compared to normal cells (Johnstone *et al.*, 2002). Despite that, studies have indicated that most classes of chemotherapeutic drugs used in cancer therapy have the ability to induce apoptosis, including the anti-metabolites (Sarraf *et al.*, 1993), DNA fragmenting (Tounekti *et al.*, 1993) and DNA crosslinking agents (Osawa *et al.*, 2011), intercalating agents (Skladanowski and Konopa, 1993), protein synthesis inhibitors (Story *et al.*, 1993), kinase inhibitors (Jacquel *et al.*, 2003) and many more.

The challenge lies in developing an effective chemotherapeutic agent that induces selective death in tumour cells, but spares the normal healthy cells (Kasibhatla and Tseng, 2003). Therefore, in this study, we attempted to elucidate the anti-cancer properties of the tocotrienol isoform, delta-tocotrienol (δ -T3) in an *in vitro* model. The K562, a cell line isolated from a CML patient was selected as a cell-based model for this study. To our knowledge, there have not been many reports outlining the function of δ -T3 in inhibiting leukemic cell growth. The objectives of this study are to: (i) investigate the cytotoxicity of δ -T3 towards K562 cells and (ii) to investigate the apoptotic genes regulated by δ -T3 that lead to K562 cell death.

METHODOLOGY

Test Compound

Stock solutions of 10 mg ml⁻¹ palm δ -T3 (kind gift from Davos Life Sciences Ptd Ltd, Singapore) was prepared in dimethyl sulfoxide (Sigma, USA) and stored at -20°C.

Cell Culture

The human chronic myeloid leukemic cells (K562 cells) were maintained in complete culture media [RPMI 1640 media (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% pen-strep (Gibco, USA)] in a humidified incubator supplied with 5% carbon dioxide (CO₂) at 37°C. Cells were sub-cultured twice weekly. These cells are non-adherent cells.

Cytotoxicity Assay

Two-fold serial dilutions of the δ -T3 stock solution (30 μ g ml⁻¹) were performed in 96 wells. The K562 cells were seeded into 96-well plates pre-loaded with δ -T3 at a density of 10 000 cells/well and cultured for 24, 48 or 72 hr at 37°C in a humidified CO₂ incubator. Untreated cells served as control. At the end of each time-point, 20 μ l of the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reagent (5 mg ml⁻¹) (Sigma, USA) was added into each well and the plate was incubated at 37°C in a humidified CO₂ incubator for 4 hr. After 4 hr, the plate containing K562 cells was centrifuged (200 g x 10 min) to pellet the formazan crystals. Following this the supernatant was aspirated and discarded. Then 100 μ l of DMSO was added to each well to solubilise the formazan crystals that were formed and the absorbance at 570 nm was read using a microplate reader (Dynex Technologies, USA). Cell viability was calculated using the following formula (Loganathan *et al.*, 2013):

$$\text{Percentage cell viability} = \left[\frac{\text{Abs}^* \text{ treated cells}}{\text{Abs}^* \text{ control cells}} \right] \times 100$$

*Abs = Absorbance of cells at 570 nm.

RNA Extraction

The K562 cells were treated with δ -T3 at their 72 hr IC₅₀ dose as obtained from the cytotoxicity assay (8.04 μ g ml⁻¹) and cultured for 72 hr at 37°C in a humidified CO₂ incubator. Untreated cells were used as control. Ribonucleic acid (RNA) was extracted from untreated and δ -T3-treated K562 cells using the RNeasy Plus Mini Kit using the manufacturer (QIAGEN, Germany) recommended protocol. The RNA samples were tested for quantity and quality as well as RNA integrity. Only RNA samples with the absorbance (Tecan, USA) at 260:230 nm ratio > 1.7 as well as absorbance at 260:280 nm ratio > 1.8 were selected for verification of RNA integrity using a bioanalyser (Agilent, USA). For the quantitative Real-time polymerase chain reaction (qPCR) analysis, only RNA samples with RNA integrity number (RIN) values more than 7 were used.

Human Apoptosis Quantitative PCR Array

The human apoptosis qPCR array (SABiosciences, USA) was run in three biological replicates for δ -T3-treated K562 cells as well as the untreated cells (negative control). Briefly, the RNA samples that passed the criteria set by manufacturer (260/230 ratio > 1.7; 260/280 ratio > 1.8 and RIN > 7) were reverse transcribed to complementary DNA (cDNA) using the RT² First Strand Kit (SABioscience, USA). The cDNA and the qPCR components were prepared with the RT²

SYBR Green Mastermix (SABiosciences, USA) and mixed with the appropriate volume of ultra-pure water before loading into the RT² Profiler PCR array's 96-well plate (SABiosciences, USA) as per manufacturer's instructions (SABiosciences, USA). The qPCR was performed in IQ5 Thermal-cycler (Biorad, USA) with the following cycling condition: One cycle of 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Then one cycle was run for 1 min at 95°C followed by another cycle at 55°C. Finally, 81 cycles were run for 10 s each at 55°C. Fold change was calculated using the $\Delta\Delta C_T$ method. The threshold cycle (C_T) values for all wells were exported into the PCR Array Data was transferred to a data analysis Excel file provided by the manufacturer (SABiosciences, USA) and data analysis was performed using a web-based PCR Array Data Analysis software that can be accessed using the Internet at the following URL: www.SABiosciences.com/pcrarraydataanalysis.php.

Data Analysis

Data was expressed as mean \pm standard deviation. The statistical test used was the one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test to distinguish differences between groups. Results were accepted as statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Cytotoxicity Assay

In anti-cancer therapy, anti-cancer agents are employed to induce cancer cell death, preferably via the induction of apoptosis (Fulda and Debatin, 2006). Therefore, it follows that the first step in determining the anti-cancer potential of δ -T3 was to determine if δ -T3 was able to induce cell death of K562 cells. The cytotoxicity of δ -T3 towards K562 cells was measured using the MTT assay. The goal of this assay was to observe and measure the cytotoxicity of δ -T3 towards K562 cells. From our results, we observed that δ -T3 caused a dose- and time-dependent reduction in K562 cell viability (Figure 1). The IC_{50} values for δ -T3-treated K562 cells were 11.65, 9.46 and 8.04 $\mu\text{g ml}^{-1}$ for 24, 48 and 72 hr respectively. To the best of our knowledge, δ -T3-induced toxicity was not reported in this cell line in previous studies. However, δ -T3 was proven effective in inducing the cell death of many cancer cell lines *in vitro* as measured by their IC_{50} values, including human liver carcinoma HepG2 cells (9.6 μM) (Wada *et al.*, 2005); human acute lymphocytic leukaemia cells CEMSS (3.8 $\mu\text{g ml}^{-1}$) (Wong *et al.*, 2012); and several pancreatic cancer cell lines Mia PaCa-2 (28 μM), PANC-1 (35 μM) and BxPC-3 (35

μM) (Hussein and Mo, 2009). Besides cytotoxicity, δ -T3 reportedly inhibited cell proliferation in the human promyelocytic leukemic cells (HL-60) at doses of 5 $\mu\text{g ml}^{-1}$ and 10 $\mu\text{g ml}^{-1}$ (de Mesquita *et al.*, 2011), which further supports the role of δ -T3 as an effective cytotoxic agent.

It was of our interest to characterise the gene expression changes that led to the induction of K562 cell death upon treatment with δ -T3. This was because many cytotoxic chemotherapeutic agents have apoptosis-inducing potential which leads to tumour cell death. Therefore, we selected a quantitative reverse transcription-polymerase chain reaction (qRT-PCR)-based gene array system to identify the gene network regulated by δ -T3 in K562 cells. The human apoptosis PCR array was a commercial kit utilised for this purpose. From the MTT assay results, we observed cell death to be highly induced following 72 hr treatment with δ -T3. Therefore, we treated K562 cells at their 72 hr IC_{50} dose, performed RNA extraction and eventually, real time PCR analysis. From the 84 genes included in the human apoptosis PCR array, δ -T3 up-regulated the expression of 24 genes related to apoptosis (Table 1). Significant gene expression changes are represented in the form of a volcano plot (Figure 2) They were the BCL-2 family (*BAD*, *BCL2L1*, *BNIP3L*, *HRK*, *MCL1*), the caspases (*CASP7*, *CASP8*, *CASP9*), the tumour necrosis factor receptor (TNFR) super family (*CD40*, *FAS*, *TNFRSF10B*, *TNFRSF1A*, *TNFRSF9*) and the P53 family (*TP53*, *TP73*). Several other genes, such as genes from the caspase recruiting domain (CARD) members (*APAF1*, *BIRC3*, *NOD1*, *NOL3* and *PYCARD*), TNF (ligand) superfamily members (*CD70* and *FASLG*), *DAPK1* and *GADD45A* were also up-regulated in K562 cells following δ -T3 treatment. The interactions between the genes regulated by δ -T3 treatment in K562 cells are shown in Figure 3.

The *TP53* gene was induced following tocotrienol-rich fraction (TRF) treatment in human colon carcinoma RKO cells (Agarwal *et al.*, 2004) and in SKBR3 breast cancer cells following exposure to δ -T3 (Pierpaoli *et al.*, 2010b). Similarly, we observed an up-regulation following *TP53* gene expression in K562 cells (Table 1). The *TP53* gene encodes the P53 tumour suppressor protein. The P53 is central to δ -T3-mediated apoptosis in K562 cells as it regulates genes such as *BCL2L1* (Lee *et al.*, 2008), *CASP8* (Liu *et al.*, 2011), *FAS* (Muller *et al.*, 1998), *GADD45A* (Xiao *et al.*, 2000), *MCL1* (Pietrzak and Puzianowska-Kuznicka, 2008) and *TNFRSF10B* (Surget *et al.*, 2012), all of which were over-expressed in the K562 cells following treatment with δ -T3. Mutations in the *TP53* gene are reported to predispose an individual to various cancers such as breast cancer (Walerych *et al.*, 2012), soft tissue sarcoma (Cordon-Cardo *et al.*, 1994), bone (Kleihues *et al.*, 1997) and brain (Sidransky *et al.*, 1992) cancers, leukaemia (Felix *et al.*,

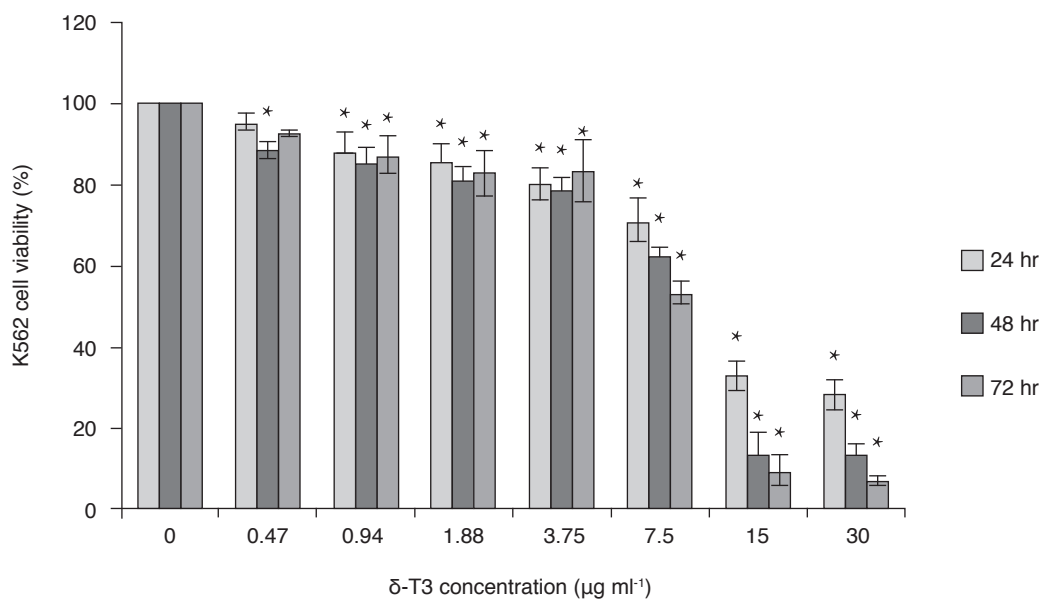


Figure 1. Dose- and time-dependent cytotoxicity of delta-tocotrienol ($\delta T3$) towards K562 cells.

TABLE 1. GENES INVOLVED IN APOPTOSIS THAT WAS DIFFERENTIALLY REGULATED IN K562 CML CELLS TREATED WITH δ -TOCOTRIENOLS

Group	Gene symbol	Gene description	Fold* change
BCL-2 family	<i>BAD</i>	BCL2-associated agonist of cell death	2.4509
	<i>BCL2L1</i>	BCL2-like 1	2.0139
	<i>BNIP3L</i>	BCL2/adenovirus E1B 19kDa interacting protein 3-like	2.6087
	<i>HRK</i>	Harakiri, BCL2 interacting protein (contains only BH3 domain)	4.7459
	<i>MCL1</i>	Myeloid cell leukemia sequence 1 (BCL2-related)	2.2449
CARD domain	<i>APAF1</i>	Apoptotic peptidase activating factor 1	2.3349
	<i>BIRC3</i>	Baculoviral IAP repeat containing 3	3.0035
	<i>NOD1</i>	Nucleotide-binding oligomerisation domain containing 1	3.0455
	<i>NOL3</i>	Nucleolar protein 3 (apoptosis repressor with CARD domain)	2.5257
Caspase family	<i>PYCARD</i>	PYD and CARD domain containing	6.8053
	<i>CASP7</i>	Caspase 7, apoptosis-related cysteine peptidase	3.5308
	<i>CASP8</i>	Caspase 8, apoptosis-related cysteine peptidase	2.2038
GADD family	<i>CASP9</i>	Caspase 9, apoptosis-related cysteine peptidase (CARD domain-containing)	2.4967
	<i>GADD45A</i>	Growth arrest and DNA-damage-inducible, alpha	2.4737
P53 family	<i>TP53</i>	Tumour protein p53	2.5257
	<i>TP73</i>	Tumour protein p73	3.249
TNF receptor superfamily	<i>CD40</i>	CD40 molecule, TNF receptor superfamily member 5	3.539
	<i>FAS</i>	Fas (TNF receptor superfamily, member 6)	2.0467
	<i>TNFRSF10B</i>	Tumour necrosis factor receptor superfamily, member 10b	3.2792
	<i>TNFRSF1A</i>	Tumour necrosis factor receptor superfamily, member 1A	2.2243
TNF (ligand) superfamily	<i>TNFRSF9</i>	Tumour necrosis factor receptor superfamily, member 9	8.2059
	<i>CD70</i>	CD70 molecule	9.4261
	<i>FASLG</i>	Fas ligand (TNF superfamily, member 6)	2.8812
	<i>DAPK1</i>	Death-associated protein kinase 1	2.1886

Note: * P<0.05.

1992) and the Li-Fraumeni Syndrome (Malkin *et al.*, 1990). Yonish-Rouach *et al.* (1991) had reported that restoring *TP53* functions in tumour cells can reverse the susceptibility to cancer as the re-introduction of wild type *TP53* gene into leukemic cells lacking this

gene induced apoptotic cell death (Yonish-Rouach *et al.*, 1991). Another member of the P53 family, the *TP73* gene was induced in K562 cells treated with δ -T3 and is commonly induced following DNA damage resulting in apoptosis (Urist *et al.*, 2004).

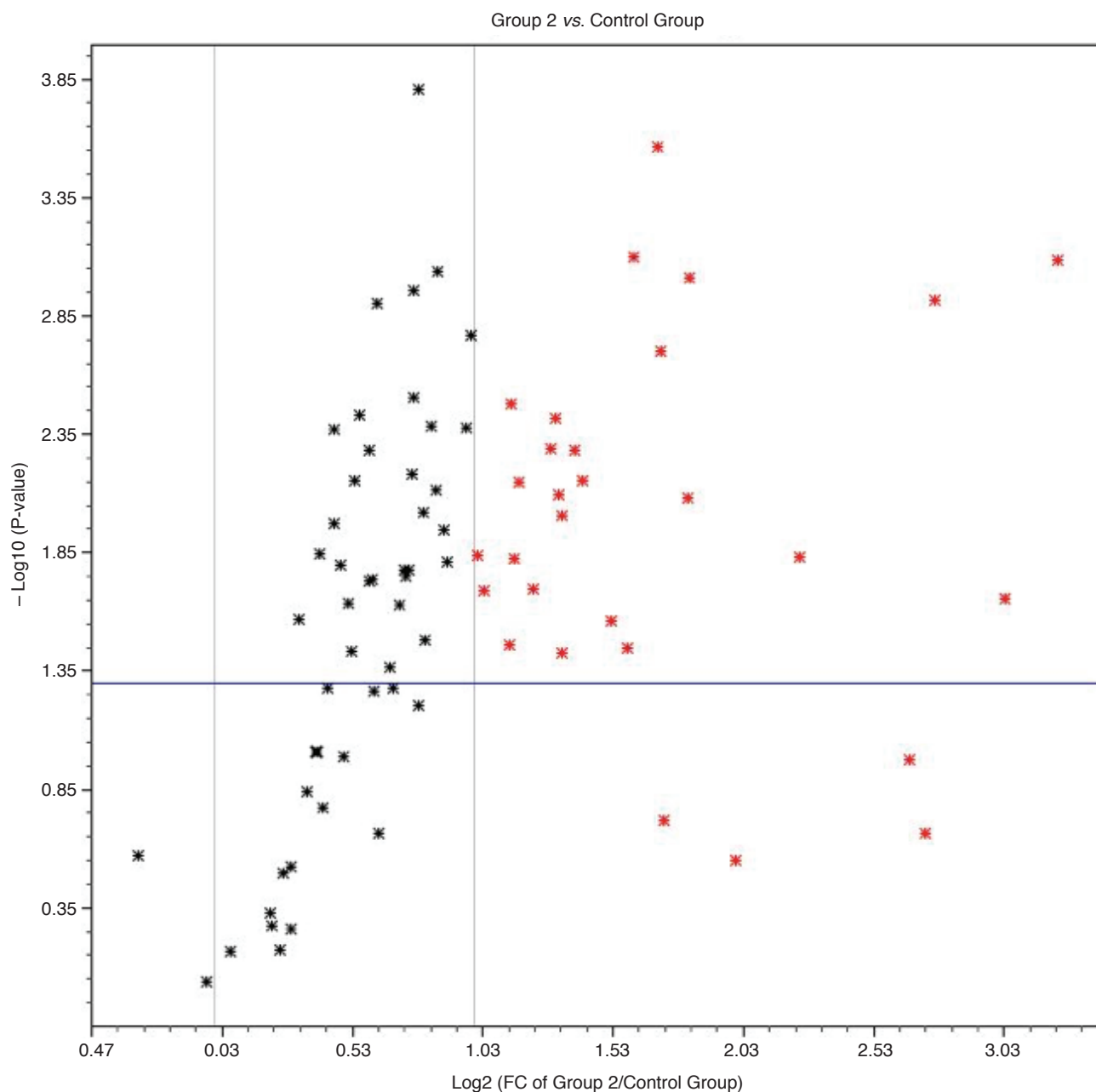


Figure 2. Volcano plot representing significant gene expression changes in K562 cells after treatment with delta-tocotrienol (δ T3). Genes with fold changes of two or more with *p*-values above 0.05 were considered significantly expressed (upper right quadrant). Real-time polymerase chain reaction (PCR) data is representative of three biological replicates.

In addition to the *TP53* gene, δ -T3 treatment also induced over-expression of the *TP73* gene in K562 cells treated with δ -T3.

Besides *TP53*, δ -T3 appears to regulate apoptosis by targeting members of the extrinsic and intrinsic apoptotic pathways by targeting the members of the tumour necrosis factor (TNF) superfamily and TNF ligand superfamily (Table 1). The TNF family and its related receptors play a key role in the extrinsic pathway of apoptosis, especially in its initiation stage (Khosravi-Far and Esposti, 2004). Several death receptor (DR) genes such as *FAS*, *TNFRSF10B* and *TNFRSF1A* genes (Lavrik *et al.*, 2005) were found to be up-regulated in the K562

cells following treatment with δ -T3. The expression of *FAS* (Wilankar *et al.*, 2011) and *TNFRSF10B* (Kannappan *et al.*, 2010) were previously reported to be induced by gamma-tocotrienol (γ T3). The *FAS* and its ligand, *FASLG* work hand in hand to bring about apoptotic cell death by the activation of the *FAS*-associated death domain (*FADD*) protein and *Caspase 8* (*CASP8*) (Nagata and Golstein, 1995). These molecules form the death-inducing signalling complex (*DISC*) (Peter and Krammer, 2003), which results in the activation of *CASP8*, thus triggering the activation of downstream effector caspases (Khosravi-Far and Esposti, 2004). The expression of *FAS* is induced by the central *TP53* protein (Figure 3),

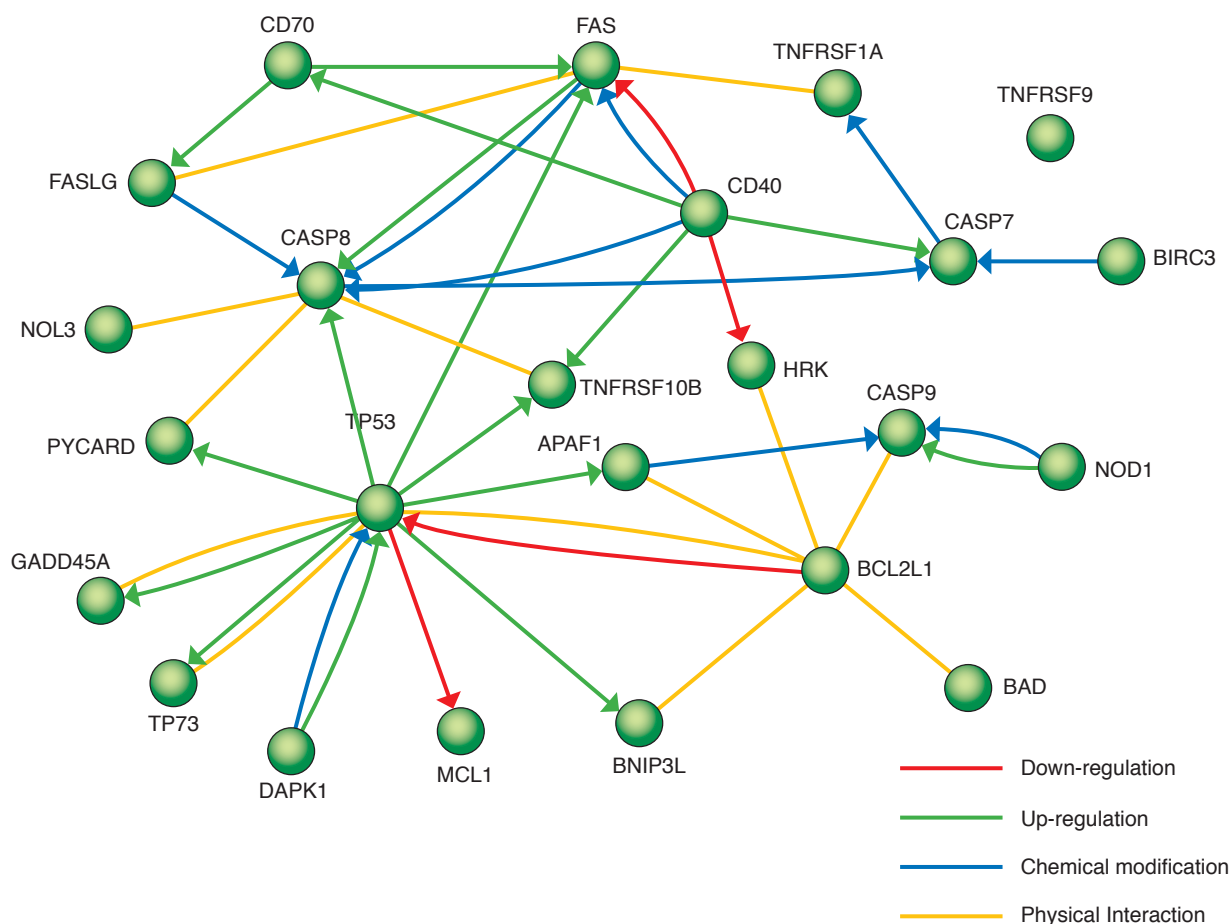


Figure 3. Gene network of apoptotic genes generated using the Gene Network Central Pro software (SABiosciences, USA) for K562 cells treated with delta-tocotrienol (δ -T3). Only genes expressed with a two-fold increase compared to untreated K562 cells are displayed in this figure.

after cellular events such as DNA damage (Muller *et al.*, 1998). In the leukemic context, FAS expression following chemotherapy has been positively associated with prolonged life span in adult acute myeloid leukaemia patients (Min *et al.*, 2004).

The protein encoded by the *TNFRSF10B* gene induces apoptotic signalling in the same manner as FAS (Lavrik *et al.*, 2005); *i.e.* through the formation of the DISC with the recruitment of FADD and subsequent activation of CASP8 (Johnstone *et al.*, 2008). However, *TNFRSF1A* has a slightly different mode of action that results in either survival (activation of NF- κ B) or the apoptotic response (death) (Jin *et al.*, 2005). The protein encoded by this gene engages in a transient complex with several proteins, *i.e.* TNFR-associated death domain (TRADD), RIP1 and TRAF2 (complex I), which triggers the nuclear factor-kappa B (NF- κ B) response (Micheau and Tschopp, 2003). Once the death domain (DD) of TRADD is dissociated from *TNFRSF1A*, it will interact with the DD of the FADD and subsequently with CASP8 (complex II) resulting in apoptosis (Micheau and Tschopp, 2003). The successful formation of complex II and subsequent

caspase 8 activation as well as low amounts of FLIP (induced by NF- κ B activation) commits the cell to apoptosis, rather than survival (Micheau and Tschopp, 2003).

The intrinsic pathway is mediated by the mitochondria, and the BCL2 family of genes are central to regulating this mode of apoptosis (Thomadaki and Scorilas, 2006). Delta-tocotrienol-treatment of K562 cells induced the expression of several members of this family, namely the *BAD*, *BCL2L1*, *BNIP3L*, *HRK* and *MCL1* genes. The BCL2 family consist of members dedicated to inducing apoptosis or maintaining survival of cells. The *BAD*, *BNIP3L* and *HRK* genes belong to the pro-apoptotic BCL-2 family (Reed, 1998). These genes encode the BH3-only proteins, which is a sequence of amino acids with the motif LXXXGD, of which X represents any amino acid (Youle and Strasser, 2008). These proteins are categorised to be strictly pro-apoptotic proteins (Gross *et al.*, 1999).

Pro-survival genes such as the *BCL2L1* (*BCL-XL*) and *MCL1* bear potential oncogenic functions (Adams and Cory, 1998). The pro-survival *MCL1* gene induced by δ -T3 treatment has

important functions in normal cells. For instance, in a murine model, deletion of the *MCL1* gene resulted in the failure of embryos to implant *in utero* (Rinkenberger *et al.*, 2000). The *MCL1* gene product is important in the immune system as it is essential to the development and maintenance of B- and T-lymphocytes (Opferman *et al.*, 2003) and haemopoietic cell development (Opferman *et al.*, 2005). This gene remains an attractive target in acute leukaemia treatment as its deletion in AML-afflicted mice cured them of the disease (Glaser *et al.*, 2012). The expression of the *MCL1* oncogene can be controlled by the action of TP53 protein as TP53 has been shown to suppress the *MCL1* promoter fragment (Pietrzak *et al.*, 2008).

The pro-survival function of *BCL2L1* is evidenced by its ability to interact with the pro-apoptotic BAX that can prevent the permeabilisation of the mitochondria, which then commits the cell to apoptosis (Billen *et al.*, 2008). Previous studies have found that δ -T3 inhibited expression of *BCL2L1* in a murine pancreatic cancer model (Husain *et al.*, 2013). However, in our study, the expression of the *BCL2L1* gene was up-regulated following δ -T3 treatment in the K562 CML cells. It has been reported that apoptosis blocked by *BCL2L1* can be restored following expression of the BAD gene as the BAD protein can selectively dimerise with the *BCL2L1* protein to release the pro-apoptotic BAX (Yang *et al.*, 1995). In this study, we found that δ -T3 treatment induced expression of BAD gene, which may explain why δ -T3 treatment induced apoptosis in the K562 cells despite the up-regulation of the *BCL2L1* gene.

The apoptosis process, whether via the intrinsic or extrinsic pathway, converges with the activation of the Caspases (Fulda and Debatin, 2006). Caspases are therefore known as the 'executioners of apoptosis' (Hengartner, 2000). Here, we show that δ -T3 treatment can activate a number of genes that code for various types of caspases (caspase 7, 8 and 9) in the K562 CML cells (Table 1). Caspase 8 and 9 are initiator caspases while caspase 7 belongs to the group of effector caspases (Li and Yuan, 2008). In literature, it was reported that there are three main effector caspases, CASP3, CASP6 and CASP7 (Li and Yuan, 2008). Briefly, in the intrinsic apoptosis pathway, the release of cytochrome-C from the mitochondria facilitates the formation of apoptosome, which consists of APAF-1 and CASP9; thus, activating the effector caspases (Brentnall *et al.*, 2013). The CASP8 is important in the extrinsic apoptotic pathway mediated by death receptors from the TNF receptor superfamily (TNFSF). Once CASP8 is activated by the formation of DISC, it will continue to activate the effector caspases triggering the caspase cascade (Lavrik *et al.*, 2005). Delta-tocotrienol has been reported to activate CASP3 in human melanoma cells (Fernandes *et al.*, 2010) and

CASP3 and CASP8 in pancreatic cancer cells (Husain *et al.*, 2011). However, we observed that only the CASP7 gene is up-regulated in K562 cells exposed to δ -T3 treatment (Table 1). Mice lacking CASP7 were reported to exhibit mild resistance to apoptosis (Lakhani *et al.*, 2006). In addition, CASP7 deficient mice when combined with CASP3 deficiency, were reported to die at birth (Lakhani *et al.*, 2006). These observations provide some concrete evidence on the combined physiological functions of CASP7 and CASP3 *in vivo*. Furthermore, CASP7 is an effective poly(adenosine diphosphate-ribose) polymerase (PARP) cleaver (Boucher *et al.*, 2012) and PARP cleavage is one of the hallmark features of apoptosis (Soldani and Scovassi, 2002).

CONCLUSION

Delta-tocotrienol induced expression of many pro-apoptotic genes (*BAD, BNIP3L, HRK, APAF1, NOD1, PYCARD, CASP7, CASP8, CASP9, GADD45A, TP53, TP73, CD40, FAS, TNFRSF10B, TNFRSF1A, TNFRSF9, CD70, FASLG* and *DAPK1*) compared to pro-survival genes (*BCL2L1, BIRC3, MCL1* and *NOL3*). We propose that due to the high ratio of pro-apoptosis genes compared to pro-survival genes induced in K562 cells, δ -T3 played a regulatory role in inducing cell suicide in K562 cells. Furthermore, δ -T3 induced expression of many genes that code for proteins involved in the extrinsic apoptosis pathway (*FAS, FASLG, CASP8, TNFRSF1A* and *TNFRSF10B*) [reviewed in Lavrik *et al.* (2005) and Elmore, 2007)] as well as the intrinsic apoptotic pathway (*APAF-1, CASP9, BCL2L1* and *BAD*) [reviewed in (Elmore, 2007)]. These findings from the current study suggest that palm δ -T3 can exert anticancer activities in chronic myeloid leukemic cells. There is a need to do further studies in animal models to substantiate these findings from the present study.

ACKNOWLEDGEMENT

The authors would like to thank MPOB for providing a grant to carry out this study as well as Davos Life Science Pte Ltd, Singapore for the kind gift of the delta-tocotrienol isoform.

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