[Frontiers in Bioscience 10, 699-709, January 1, 2005]

MECHANISMS MEDIATING THE ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS OF VITAMIN E IN MAMMARY CANCER CELLS

Paul W. Sylvester and Sumit J. Shah

School of Pharmacy, 700 University Avenue, University of Louisiana at Monroe, Monroe, LA 71209-0470

TABLE OF CONTENTS

1. ABSTRACT

Tocopherols and tocotrienol represent the two subgroups within the vitamin E family of compounds, but only tocotrienols display potent anticancer activity at doses that have little or no effect on normal cell growth or function. Tocotrienols are potent antioxidants, but antitumor activity is independent of antioxidant activity. The exact reason why tocotrienols are more potent than tocopherols is not completely understood, but at least part of the reason is because of greater cellular accumulation. Furthermore, dose-response studies show that growth inhibitory doses of tocotrienols are 5-6 times lower than their corresponding lethal doses, suggesting that the antiproliferative and cytotoxic effects of tocotrienols are mediated through different mechanisms. Recent studies showed that tocotrienol-induced programmed cell death (apoptosis) results from the activation of specific intracellular cysteine proteases (caspases) associated with death receptor activation and signal transduction. Furthermore, combined treatment with specific caspase inhibitors blocked the cytotoxic effects of tocotrienols in malignant mammary epithelial cells. In contrast, tocotrienol inhibition of cell proliferation appears to involve the suppression of multiple hormone- and growth factor-receptor mitogenic signaling pathways. Although additional studies are required to clarify the intracellular mechanisms mediating the anticancer effects of tocotrienols, experimental evidence strongly suggests that dietary supplementation of tocotrienols may provide significant health benefits in lowering the risk of breast cancer in women.

2. INTRODUCTION

Breast cancer is the most prevalent malignancy in women in the world (1). Although major advances have been made in early detection and treatment, breast cancer mortality is similar today as it was fifty years ago (1). More alarming is the fact that the incidence of breast cancer has increased steadily over the past 50 years (1). While specific genes such as BRCA1 and BRCA2 have been identified which predisposes women to breast cancer, these genes are estimated to be directly responsible for only 10-15% of all breast cancers (2). The cause for the remaining 85-90% breast cancers in women is unknown. Therefore, it is evident that advances in the area of breast cancer prevention would be of great benefit in significantly reducing breast cancer risk and mortality. Breast carcinogenesis is a multistage process that is initiated by a single genomic mutation, and subsequent mutations leads to a progression in malignant phenotypic characteristics, including increased anaplastic histological morphology, resistance to anticancer or endocrine therapy, and enhanced invasive and metastatic potential (3-12). Experimental evidence suggests that the dietary supplementation with specific forms of vitamin E can provide significant protection at multiple stages of mammary carcinogenesis (13-18). Since cellular transformation and tumor progression usually occurs prior to tumor detection, understanding the mechanisms mediating the anticancer effects of specific vitamin E compounds would provide essential information necessary for evaluating the potential therapeutic value that may result from dietary supplementation of these compound in reducing breast cancer incidence and mortality.

Table 1. Vitamin E Levels (mg/L) in Common Dietary Oils

Dietary Oil	Alpha-tocopherol	Alpha-tocotrienol	Gamma-tocotrienol	Delta-tocotrienol	Total Tocotrienol
Palm	152	205	439	94	738
Rice Bran	324	236	349	---	586
Wheat Germ	133	26	---	---	26
Coconut	5	5	1	19	25
Palm Kernel	12	21	---	---	21
Coco Butter	11	2	---	---	2
Corn	112	---	---	---	0
Cottonseed	389	---	---	---	0
Peanut	130	---	---	---	0
Olive	51	---	---	---	0
Safflower	387	---	---	---	0
Soybean	101	---	---	---	0
Sunflower	487	---	---	---	0

Selected information obtained from references (18) and (19).

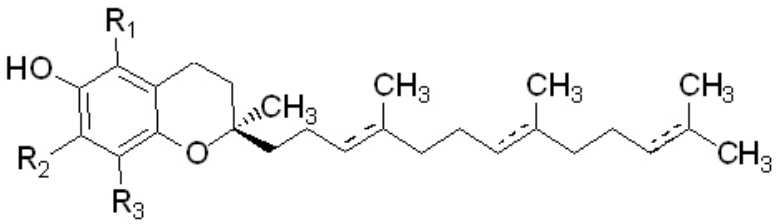
Dietary intake of vitamin E comes primarily from the consumption of vegetable oils and fats. All vitamin E compounds are potent natural antioxidants that act to prevent the spoilage, maintain flavor and enhance the nutritional value of dietary fat. However, natural antioxidants also provide significant health benefits by preventing the damaging affects of peroxidation reactions and free radical production within the body (19-21). Uncontrolled production of free radicals is associated with damage to cell structures, reduced cellular function, and implicated as a cause of various diseases, such as arteriosclerosis and cancer (19-21). Palm oil is unique compared to other dietary fats in that palm oil contains the highest known concentrations of natural antioxidants, particularly vitamin E (17, 18, 22, 23). Although vitamin E contributes to the maintenance of good health and disease prevention, palm vitamin E is unique as it contains 70-80% tocotrienols (Table 1), a rare form of vitamin E that displays potent anticancer activity. The present review will attempt to summarize the present literature and clarify the intracellular signaling mechanisms involved in mediating the anticancer effects of tocotrienols.

3. VITAMIN E COMPOUNDS

Vitamin E is a generic term that refers to family of compounds that are further divided into two subgroups called tocopherols and tocotrienols. Tocopherols and tocotrienols have the same basic chemical structure characterized by a long phytyl chain attached at the 1-position of a chromane ring structure (figure 1). However, tocopherols have a saturated, while tocotrienols have an unsaturated phytyl chain (figure 1). Each subgroup of vitamin E contains several isoforms and individual tocopherols and tocotrienols isoforms differ from each other based on the number of methyl groups bound to their chromane ring. Although very similar in their chemical structure, numerous investigations have clearly demonstrated that individual tocotrienol isoforms display significantly greater anticancer activity than their corresponding tocopherol isoforms (24). These findings are particularly interesting because they are observed using treatment doses that have little or no effect on cell growth or viability (25). Furthermore, dose-response studies also showed that treatment doses that inhibited cell proliferation after 5 days in culture by 50% (IC_{50}) for individual tocotrienol isoforms were 5-6 times lower than their corresponding cytotoxic doses that induced 50% cell death (LD_{50}) after a 24 hr treatment exposure period (24, 25). These data strongly suggested that the antiproliferative and apoptotic effects of tocotrienols are mediated through different mechanisms. These and other studies investigating the intracellular mechanisms responsible for mediating antiproliferative and cytotoxic effects of tocotrienols have provided strong experimental evidence to suggest that dietary supplementation of tocotrienols may provide significant health benefits in preventing or reducing the risk of breast cancer in women.

4. TOCOTRIENOLS AS ANTICANCER AGENTS

Initially, the anticancer effects of tocotrienols were discovered in studies investigating the effects of high dietary fat intake on mammary tumorigenesis in laboratory animals. Studies clearly demonstrated that consumption of high fat diets significantly stimulates carcinogen-induced mammary tumor development and growth in rats (7, 16). Subsequent studies tried to determine if this phenomenon was due simply to some nonspecific metabolic effect resulting from the consumption high levels of fat calories or specifically associated with the type of fat consumed (16). In general, these studies showed that consumption of high fat diets stimulated murine mammary tumorigenesis regardless whether the diets were formulated with animal or vegetable fats, or composed of varying amounts of saturated versus unsaturated fats (16). A notable exception to this finding was the observation that high dietary intake of palm oil, in contrast to other high fat diets formulated from other types of vegetable oils, suppressed carcinogen-induced mammary tumorigenesis in experimental animals (16**).** Table 1 shows that palm oil differs from other dietary lipids in that it naturally contains very high levels of a rare form of vitamin E called tocotrienol (17, 18, 22, 23). In addition, studies also showed that consumption of high palm oil diets stripped of tocotrienols, no longer provided a protective effect against mammary tumorigenesis in these animal (26). These data were the first experimental evidence of the anticancer activity of tocotrienols in laboratory animals and suggested that this particular form of vitamin E may have potential use as a chemotherapeutic agent in the prevention and/or treatment of breast cancer in women.



Compound	R_1	R_2	R_3	Phytyl Chain
alpha-tocopherol	CH_3	CH_3	CH_3	Saturated
gamma-tocopherol	H	CH_3	CH_3	Saturated
beta-tocopherol	CH_3	H	CH_3	Saturated
delta-tocopherol	H	H	CH_3	Saturated
alpha-tocotrienol	CH_3	CH_3	CH_3	Unsaturated
gamma-tocotrienol	H	CH_3	CH_3	Unsaturated
beta-tocotrienol	CH_3	H	CH_3	Saturated
delta-tocotrienol	H	H	CH_3	Unsaturated

Figure 1. The basic chemical structure of vitamin E compounds. The only difference between tocopherols and tocotrienols, the two subgroups within the vitamin E family of compounds, is that tocopherols have a saturated, whereas tocotrienols have an unsaturated phytyl tail connected at the 1-position of a chromane ring structure. Different isoforms within each subgroup differ from each other based on the degree of chromane ring methylation.

5. ANTIOXIDANT POTENCY OF TOCOTRIENOLS

Tocopherols and tocotrienols contain a phenolic hydroxyl group located on the number 6 carbon of the chromane ring, which functions as the reactive site that is responsible for mediating the antioxidant activity of these compounds. Although tocopherols and tocotrienols are both potent antioxidants that inhibit peroxidation reactions and controls free-radical production within the body (19), direct comparisons between specific tocopherol and tocotrienol isoforms have shown large differences in antioxidant activity. Studies showed that alpha-tocotrienol displayed between 40-60 times greater potency in preventing lipid peroxidation in rat liver microsomal membranes and 6-7 times greater activity in preventing cytochrome P-450 oxidative damage as compared to alpha-tocopherol (27-29). The significantly greater antioxidant potency of tocotrienols versus tocopherols results from several factors. First, tocotrienols display a much greater recycling efficiency than tocopherols (27-29). Furthermore, tocotrienols display a uniform distribution within the microsomal membrane lipid bilayer and a more efficient interaction with lipid free radicals, as compared to tocopherols (27-29). Nevertheless, although tocotrienols display significantly more potent antioxidants than tocopherols, the majority of the anticancer effects induced by tocotrienols are not associated with antioxidant activity or potency (13, 20, 21, 26, 30, 31).

6. DIFFERENTIAL TARGET TISSUE UPTAKE OF TOCOPHEROLS AND TOCOTRIENOLS

Individual tocopherols and tocotrienol isomers display great differences in absorption transport in the blood and lymph, and tissue accumulation (32-34). In most tissues, alpha-tocopherol is found in the highest concentrations due to the presence of a specific binding protein that binds and retains the alpha-isomer in the cell (32-34). Different cell types characteristically contain different levels of alpha-tocopherol binding protein (33, 34). In contrast, no such isoform specific binding protein has been shown to exist for tocotrienols and cellular accumulation of tocotrienols appears to be mediated by nonspecific mechanisms. It is important to note that in order for dietary lipids and fat-soluble vitamins to be absorbed from the gastrointestinal tract, they must first be emulsified by bile and packaged into micelles for transport into the circulation (35-37). Furthermore, bile excretions is stimulated by the amount and type of fat consumed in the diet. Studies have shown that tocotrienol absorption is significantly reduced if taken by fasted individuals as compared to individuals who ingest tocotrienols during or immediately after a full meal (38, 39).

Similarly, all vitamin E compounds are extremely lipophilic and must be transported in the blood by lipoproteins (35-37). Differences in the levels and types of specific apolipoproteins located on the surface of specific lipoproteins significantly influences carrier protein interaction with target tissues and activation of target tissue cell membrane receptors and enzymes (35-37). Tocopherols and tocotrienols are transported in the blood by different classes of lipoproteins that display different target tissue specificity (35-37). Tocopherols are primarily carried in cholesterol-rich low-density (LDL) and high-density (HDL) lipoproteins, whereas tocotrienols are mainly transported in triglyceride-rich chylomicrons (35-37). Target tissue sites for LDL lipoproteins include the liver, kidney, and spleen among others, while chylomicrons primarily transport triglycerides to the adipose tissue.

Since tocotrienols accumulate in adipose tissue such as the mammary gland fat pad, it is possible that high levels of tocotrienols within the microenvironment of the mammary gland may act to inhibit neoplastic mammary epithelial cells growth and survival. However, it is also well established that tocopherol and tocotrienol plasma and tissue concentrations is limited by the specificity and saturability of specific transfer proteins and transport mechanisms with in the body that show significant preference for alpha-tocopherol (32, 38). Consumption of large amounts of alpha-tocopherol in combination with tocotrienols has been shown to competitively inhibit tocotrienol absorption from the diet (32, 38).

7. RELATIVE ANTICANCER POTENCY OF INDIVIDUAL TOCOPHEROLS AND TOCOTRIENOLS

The anticancer effects of tocotrienols are well established *in vitro* using the tocotrienol-rich-fraction (TRF) of palm oils (24, 40-43). TRF is obtained from the extraction of all forms of vitamin E from palm oil. The composition of TRF is approximately 20.2% alpha-tocopherol, 16.8% alpha-tocotrienol, 44.9% gamma-tocotrienol, 14.8% delta-tocotrienol, and 3.2% of a non-vitamin E lipid soluble contaminant (24, 40-43). Initial studies showed that supplementation of culture media with 0-120 microM TRF significantly inhibited mammary tumor cell proliferation and induced cell death in a dose-responsive manner (24). Since TRF contains a mixture of alpha-tocopherol and alpha-, gamma-, and delta-tocotrienol, it was unclear if the anticancer effects of TRF were mediated one or all of these vitamin E isoforms. Direct comparisons between individual tocopherol and tocotrienol isoforms showed that tocotrienols were significantly more potent that tocopherols (24, 25). Furthermore, the relative antiproliferative and apoptotic biopotency of specific isoforms displays a consistent relationship corresponding to delta-tocotrienol $\geq$ gamma-tocotrienol > alpha-tocotrienol > delta-tocopherol >> gamma- and delta-tocopherol (24, 25, 40). Furthermore, cells with the greatest degree of malignancy also displayed the greatest sensitivity to the antiproliferative and apoptotic actions of tocotrienols, as compared to normal mammary epithelial cells (24, 25). One reason explaining the greater biopotency of tocotrienols is the finding that tocotrienols are more easily accumulated or taken up into normal and neoplastic mammary epithelial cells than tocopherols (24, 25). It is believed that the unsaturated phytyl chain produces a less planar molecular conformation that facilitates less restricted transmembrane passage of tocotrienols into the cell (24, 25). Since there is greater uptake, there are higher concentrations of tocotrienols at intracellular sites of action and are thereby able to induce a greater biological response. However, treatments that produce comparable intracellular levels of tocopherols did not elicit the same antiproliferative and apoptotic effects as tocotrienols (24, 25). These findings demonstrate that tocotrienols are inherently more potent than tocopherols in antiproliferative and apoptotic activity. Some studies have shown that treatment with very high doses of alpha-tocopherol in the millimolar range was also found to inhibit cell growth and induce apoptosis in various cell types (18, 34). However, the physiological significance of these studies is not clearly evident.

8. TOCOTRIENOL EFFECTS ON EGF-RECEPTOR MITOGENIC SIGNALING

Initial studies demonstrated that moderate treatment doses of tocotrienols significantly inhibited EGF-dependent normal, preneoplastic, and neoplastic mammary epithelial cell proliferation in a dose-dependent manner *in vitro* (24, 25). The mitogenic actions of EGF are mediated through specific membrane-bound EGF-receptors that contain a cytoplasmic tyrosine kinase domain (44). EGF stimulation of the EGF-receptor activates receptor tyrosine kinase activity and induces EGF-receptor autophosphorylation (44). EGF-receptor autophosphorylation sites are required for substrate interaction and tyrosine phosphorylation (44). However, tocopherol and tocotrienol suppression of EGF-dependent mitogenesis was not found to be associated with a reduction in EGF-receptor levels or tyrosine kinase activity function in these cells (40, 41). These findings indicate that the antiproliferative effects of specific tocopherol and tocotrienol isoforms do not result from suppression in EGF-receptor mitogenic responsiveness. Instead, the antiproliferative effects of tocotrienol on EGF-dependent cell growth occur downstream from the EGF-receptor.

9. TOCOTRIENOL INHIBITION OF PROTEIN KINASE C ACTIVITY

It is well established that EGF stimulation of the EGF-receptor initiates multiple signal transduction pathways associated with cell proliferation, including PKC activation (45-47). Treatment with phorbol ester has been shown to enhance phospholipid-dependent PKC activation and potentiate EGF-dependent mammary epithelial cell growth, whereas treatment with PKC inhibitors induces the opposite results (45-47),b). PKC consist of a large family of structurally homologous serine/threonine kinases, which display a wide range of tissue and cellular distribution and have distinct sensitivities to lipid activation and regulation (47)). Previous studies have shown that PKCalpha is present in the highest concentrations in normal mammary epithelial cells and the relative levels of this PKC isoform increases throughout the period of log phase growth (47)). Studies showed that growth inhibitory doses of specific tocopherol and tocotrienol isoforms were found to have little or no effect on total intracellular levels of PKCalpha in normal mammary epithelial cells grown in primary culture (40). However, these same treatments were observed to inhibit EGF-induced PKCalpha translocation from the cytosolic to membrane fraction. In contrast, treatment with relatively high doses (250 microM) of alpha- or gamma-tocopherol had no effect on EGF-dependent mammary epithelial cell proliferation or PKCalpha translocation (40). Previous investigations in vascular smooth muscle cells have shown that alpha-tocopherol, but not beta-tocopherol, indirectly attenuates PKCalpha activation by stimulating protein phosphatases

that act to dephosphorylate and diminish PKCalpha enzymatic activity (48, 49). These data suggest that tocotrienol-induced inhibition of EGF-dependent normal mammary cell proliferation is due, at least in part, to a reduction in PKCalpha activation and translocation from the cytosolic to membrane fraction.

10. TOCOTRIENOL INHIBITION OF G-PROTEIN MITOGENIC SIGNALING

It is well established that activation of the EGF-receptor can initiate multiple cAMP-dependent mitogenic signaling pathways. EGF activation of the G-protein coupled EGF-receptor stimulates adenylyl cyclase, thereby elevating in intracellular cAMP levels (45, 50-52). Increased cAMP production will activate guanine nucleotide exchange factors (cAMP-GEFs), such as Rap-Ras proteins that then activate Raf proteins, and subsequently lead to the activation of the MAPK mitogenic signaling cascade (44, 52). The EGF-receptor stimulation of cAMP-GEFs and/or direct tyrosine phosphorylation of phosphoinositide 3-kinase (PI3K) can also lead to the activation PI3K-dependent kinase (PDK), which phosphorylates and activates Akt (52). Activation of Akt subsequently leads to the phosphorylation and regulation of various targets involved in mitogenic signaling and cell survival, including transcription factors such as forkhead and Daf 12 (52-54). Studies showed that treatment of preneoplastic mammary epithelial cells with growth inhibitory doses of tocotrienols resulted in a reduction in both MAPK and Akt activation (41). Furthermore, that combined treatment with pharmacological agents that either enhance G protein function (cholera and pertussis toxin) or increase intracellular cAMP levels (forskolin and 8-Br-cAMP) completely reversed the growth inhibitory effects of tocotrienols on preneoplastic mammary epithelial cells (41). These findings strongly suggest that tocotrienols indirectly attenuate EGF-dependent MAPK and PI3K/Akt mitogenic signaling by inhibiting early post-EGF-receptor upstream events involved in cAMP production in preneoplastic mammary epithelial cells *in vitro*.

11. TOCOTRIENOL INHIBITION OF PI3K/PDK/AKT MITOGENIC SIGNALING

Recent studies in highly malignant mammary epithelial cells also showed that the antiproliferative effects of gamma-tocotrienol are associated with a decrease in the phosphorylation and activation of PDK1 and Akt (55). These studies also showed that tocotrienol-induced reductions in PDK1 and Akt occurred independently of changes in PTEN or PP2A phosphatase activity. These studies also showed that tocotrienol treatment decreased NFkappaB transcriptional activity. NFkappaB proteins constitute an inducible family of transcription factors that have been implicated in the regulation of cell proliferation, cell survival, tumor development as well as malignant transformation (56-59). EGF-induced activation of the PI-3K/PDK-1/Akt mitogenic pathway has been shown to enhance NFkappaB transcriptional activity (57, 58). NFkappaB exists as a heterodimer composed of the p65 and p50 subunits, and it is the p65 subunit that contains the transcriptional activation domain required for initiating gene transcription (56). The IkappaB inhibitory proteins bind to NFkappaB and suppress transcriptional activity by preventing its translocation for the cytoplasm to the nucleus (56). EGF-dependent activation of PI3K and Akt has been shown to stimulate the activation of IkappaB kinase (IKK), an enzyme that phosphorylates IkappaB, which promotes IkappaB degradation and subsequently NFkappaB activation (56, 58, 60). Experimental evidence suggests that the inhibitory effects of tocotrienol on NFkappaB activation, appear to result from a reduction in IKK-alpha/betaand enhanced IkappaB binding and suppression of NFkappaB transcriptional activity. Since Akt and NFkappaB play a critical role in development and progression of mammary tumorigenesis, and overexpression of activated Akt and NFkappaB is associated with the development of resistance to chemotherapy in a majority of breast cancer cases (56, 58-60), these findings suggest that tocotrienols may have potential value as a chemotherapeutic agent against mutlidrug resistant forms of breast cancer.

12. TOCOTRIENOL-INDUCED APOPTOSIS

Previous studies have shown that treatment with high doses of tocotrienols induced apoptosis in normal, preneoplastic and neoplastic mammary epithelial cells (24,25). Apoptosis is an important aspect of normal mammary gland growth and remodeling, as well as a mechanism for eliminating neoplastic cells from the breast (61). Apoptosis is characterized by distinct morphological and biochemical features, such as nuclear and cytoplasmic condensation, DNA fragmentation, dilation of the endoplasmic reticulum, alterations in the cell membrane composition, and formation of membrane-enclosed apoptotic bodies (62). Initiation of apoptosis involves the activation of cysteine-dependent aspartyl proteases known as caspases (63-66). Caspases are constitutively present in cells in an inactive precursor form that must then be cleaved and processed for activation (63-66). Initiator caspases (caspases-8, and -9) activate effector caspases (caspases-3, -6 and -7), which then cleave structural and regulatory proteins such as DNA fragmentation factor-45 (DFF45), poly (ADP-ribose) polymerase (PARP), lamins, and cytokeratins, and ultimately result in the organized destruction of the cell (63-66).

There are at least two general mechanisms involved in initiating caspase activation. The first mechanism involves receptor-mediated caspase activation. Activation of “death receptors”, such as Fas, tumor necrosis factor (TNF), or TNF-related apoptosis-inducing ligand (TRAIL) receptors by their specific ligands results in receptor trimerization, recruitment of adapter proteins such as Fas-associated death domain (FADD), and inactive initiator caspases (procaspase-2 or –8), to form what is called a death inducing signaling complex or DISC (67). The initiator caspase within the DISC is then activated and released. Activated initiator caspases then activate effector caspases, such as capase-3, which then mediate the various cytoplasmic and nuclear events associated with apoptosis (67). However, Initiator caspase activation can also occur

in the absence of death receptor activation. Studies have shown that stimulation of the PI3K/PDK/Akt mitogenic signaling pathway results in the increased intracellular expression of FLICE-inhibitory protein (FLIP), a cytoplasmic protein that prevents procaspase-8 activation (68-70). Treatments that inhibit PI3K/PDK/Akt mitogenic signaling can cause a reduction in intracellular FLIP levels, and a corresponding increase in caspase-8 activation and apoptosis (68-70).

In contrast, mitochondrial stress-induced caspase activation can be initiated by numerous cellular signals that cause perturbations in mitochondria resulting in the loss of mitochondrial membrane potential and release of proapoptotic molecules such as apoptosis inducing factor (AIF) and cytochrome c from the intermembrane space into the cytoplasm (71, 72). Cytochrome c then interacts with apoptosis protease activation factor-1 (Apaf-1), dATP/ATP and procaspase-9 to form a complex (apoptosome) that induces the activation of initiator caspase-9, and finally leads to the activation of effector caspase-3 and apoptosis (71, 72). Cytochrome c release from the mitochondria is required for apoptosome formation and is tightly controlled by the Bcl-2 family of proteins that function to inhibit (Bcl-2, Bcl-x_L, Bcl-w, Mcl-1 and A1) or promote (Bax, Bak, Bok, Bik, Hrk, Bim, Bid and Bad) apoptosis through the regulation of cytochrome c release (72-76). It has also been shown that cross talk between the receptor-mediated and mitochondrial stress pathway can occur in some apoptotic models. Activation of caspase-8 or downstream effector caspases can also directly lead to the release of cytochrome c from the mitochondria and caspase-9 activation in some cells (72-76).

13. TOCOTRIENOL-INDUCED CASPASE ACTIVATION

Studies showed that tocotrienol-induced apoptosis in highly malignant mammary epithelial cells was mediated by specific intracellular signaling mechanisms that increased intracellular caspase-8 and caspase-3 levels and activity (43, 55). Furthermore, it was also shown that tocotrienol-induced apoptosis is blocked by combined treatment with specific caspase-8 or caspase-3 inhibitors (43). Results also showed that tocotrienol-induced programmed cell death occurs independently of caspase-9 activation in malignant +SA mammary epithelial cells (43). Treatment with cytotoxic doses of TRF or gamma-tocotrienol had no inhibitor did not block tocotrienol-induced apoptosis in these cells (43). Since caspase-9 activation is primarily associated with mitochondrial stress-mediated apoptosis (72-76), these findings suggested that tocotrienol-induced apoptosis in malignant mammary epithelial cells is independent of mitochondrial stress-mediated signaling mechanisms. Furthermore, since caspase-8 processing and activation is associated with "death receptor" mediated apoptotic signaling (68-70), experimental evidence strongly indicated that tocotrienol-induced apoptosis is mediated by receptor-induced caspase activation. However, the specific death receptor(s) and ligand(s) that mediate tocotrienol-induced caspase activation and apoptosis have not yet been determined in these studies (42, 43, 77).

14. TOCOTRIENOL EFFECTS ON DEATH RECEPTOR APOPTOTIC SIGNALING

It is well established that Fas ligand (FasL) activation of the death receptor, Fas, stimulates procaspase-8 activation to caspase-8 (67, 78), and Fas activation has been shown to play an important role in normal mammary gland remodeling and involution, as well as, the removal of neoplastic mammary epithelial cells during the early stages of tumorigenesis (61). In addition, treatment with the succinate derivative of RRR-alpha-tocopherol, RRR-alpha-tocopheryl succinate, has been shown to induce DNA synthesis arrest and apoptosis in breast cancer cells, and these effects are associated with Fas apoptotic signaling (79, 80). However, tocotrienol treatment was found to induce apoptosis in neoplastic mammary epithelial cells that contained nonfunctional death receptors, and tocotrienol-induced caspase-8 and -3 activation occurs independently of Fas activation and apoptotic signaling (55). Treatment with high doses of Fas activation antibody or FasL alone did not induce apoptosis in these cells and had not effect on intracellular Fas, FasL and FADD levels (55). Other studies have shown that RRR-alpha-tocopheryl succinate restores Fas sensitivity and apoptotic signaling in Fas insensitive human breast cancer cells (80). However, similar treatments with tocotrienols does not restore apoptotic signaling in Fas insensitive malignant mammary epithelial cells, as indicated by the absence of FasL and FADD translocation from the cytosolic to membrane fraction (55). Likewise, treatment with high doses of with other death receptor ligands, such as TNFalpha or TRAIL, was also not found to have any adverse effect on these neoplastic mammary epithelial cells viability, suggesting that tocotrienol-induced caspase activation in these cells occurs independently of TNF-R and TRAIL-R apoptotic signaling (55).

Resistance to death receptor induced apoptosis is associated with enhanced tumorigenesis, multi-drug resistance, and enhanced survival in a number of tumor cell types (80-82). Furthermore, resistance to apoptotic stimuli can be acquired through up-regulation and enhanced activity of various mitogen-dependent signaling pathways, particularly the PI3K/PDK/Akt mitogenic signaling pathway (68-70, 83-90). Studies have shown that stimulation of PI3K/PDK/Akt mitogenic pathway enhances the expression of the anti-apoptotic protein, FLIP, which inhibits procaspase-8 cleavage and activation (68-70, 85-90). Previous investigations have also shown that over expression of FLIP is associated with tumor cell resistance to death receptor activation and apoptotic signaling, and that the ratio between caspase-8 and FLIP levels is critical in determining tumor cell sensitivity to apoptotic stimuli (91-96). Additional studies showed that tocotrienol-induced apoptosis in neoplastic mammary epithelial cells containing nonfunctional death receptors was associated with a large and prolonged decrease in intracellular FLIP levels, and a corresponding increase in intracellular cleaved (active) caspase-8 levels (55). These studies also showed that tocotrienol-induced reductions in FLIP were also associated with a large decrease in phospho-PDK-1 (active form), phospho-Akt (active form), a reduction in Akt

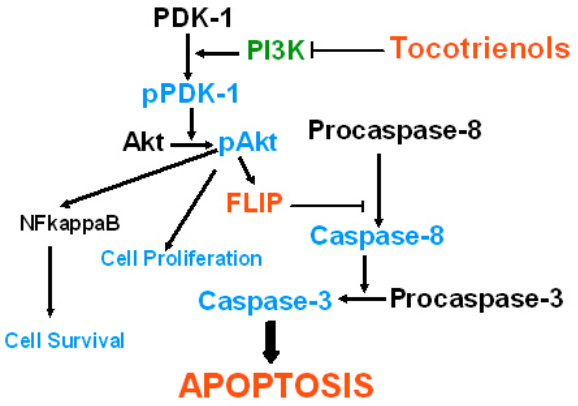


Figure 2. Intracellular target sites of action that appear to mediate the antiproliferative and apoptotic effects of tocotrienols. Tocotrienol-dependent caspase-8 activation and apoptosis in neoplastic mammary epithelial cells containing nonfunctional death receptors appears to result tocotrienol-induced suppression of PI3K/PDK/Akt signaling pathways and subsequent FLIP expression, an intracellular protein that prevents caspase-8 activation. Tocotrienol-induced inhibition of the PI3K/PDK/Akt mitogenic signaling pathways and NFkappaB activity also appears to be involved in suppressing cell proliferation and cellular survival.

kinase activity (55). At present, it is not known whether gamma-tocotrienol directly inhibits PDK-1 activity or acts at some upstream site to inhibit PDK-1 activation, possibly by suppression the activity of PI3K. In summary, these studies showed that tocotrienol-induced caspase-8 activation and apoptosis occurs independently of death receptor signaling in malignant mammary epithelial cells, and appears to result form the suppression of PI3K/PDK-1/Akt mitogenic signaling and a corresponding decrease in intracellular FLIP expression, an endogenous inhibitor of caspase-8 activation. These effects of tocotrienol on mitogenic and apoptotic pathways are summarized in figure 2.

15. TOCOTRIENOL EFFECTS ON MITOCHONDRIAL STRESS APOPTOTIC SIGNALING

Although the experimental evidence described above strongly suggest that the cytotoxic effects of tocotrienols results from death receptor activation, the specific effects of tocotrienols on mitochondrial stress apoptotic signaling was not directly investigated in these studies. Subsequent studies determined the effects of tocotrienols on the relative levels of specific anti- and pro-apoptotic mitochondrial proteins, as well as mitochondrial membrane integrity and cytochrome c release in neoplastic mammary epithelial cells grown in culture (97). Results from these studies clearly demonstrate that tocotrienol-induced programmed cell death occurs independently of mitochondrial stress apoptotic signaling in these cells. Tocotrienol-induced apoptosis was not associated with a disruption or loss of mitochondrial membrane potential or the release of cytochrome c from the mitochondria into the cytoplasm in these cells (97). Furthermore, neoplastic mammary epithelial cells undergoing tocotrienol-induced apoptosis displayed a paradoxical decrease in mitochondrial levels of pro-apoptotic proteins Bid, Bax and Bad, and a corresponding increase in mitochondrial levels of anti-apoptotic proteins, Bcl-2 and Bcl-x_L (97). These findings suggest that mitochondrial membrane stability and integrity might actually be enhanced for a limited period of time following acute tocotrienol exposure, thereby acting to inhibit mitochondrial cytochrome c release into the cytoplasm and prevent mitochondrial stress mediated apoptotic signaling in these cells. In summary, these findings clearly demonstrate that tocotrienol-induced apoptosis occurs independently of mitochondrial stress apoptotic signaling in neoplastic mammary epithelial cells.

16. CONCLUSIONS

Breast cancer is a serious problem and it is clearly evident that advances in the area of breast cancer prevention would be of great benefit in significantly reducing breast cancer risk and mortality in women. Dietary supplementation with antioxidants, particularly vitamin E is associated with providing protection against free-radial cellular damage that can lead to cellular dysfunction and death. However, it has become increasingly clear that many of the biological effects of vitamin E are mediated independently of its antioxidant activity. Furthermore, individual members within the different vitamin E subgroups display a wide variation in their antioxidant and anticancer activity. Specifically, tocotrienols display significantly greater antiproliferative and apoptotic activity than tocopherols in a variety of experimental cancer models, suggesting that dietary supplementation of tocotrienols may prevent or lower the risk of beast cancer in women. However, studies in animals and humans have produced conflicting and inconsistent results. Since alpha-tocopherol is preferentially takes up and distributes over tocotrienols in the mammalian body, it has been difficult to optimized dosage levels and absorption in the diet. More extensive research is needed in the areas related to tocotrienol pharmaceutics and pharmacokinetics in order to optimized tocotrienol formulation, dosage and delivery. Important information obtained from pharmaceutics studies could then be used to conduct meaningful and highly controlled long-term intervention studies in humans. Furthermore, additional studies are needed to understand the exact intracellular mechanism involved in mediating the antiproliferative and apoptotic effects of tocotrienols. Additional studies using modern techniques such as microarray expression profiling will provide important new information regarding the transcriptional response of breast tissue to tocotrienol treatment, and provide a solid scientific basis for highly controlled chemoprevention studies in humans.

17. ACKNOWLEDGEMENTS

This work was performed at the School of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209-0470 and supported by a grant from the NIH (Grant CA86833). All research and submission of this manuscript for review was approved by the appropriate authorities at the University of Louisiana at Monroe where the work was

carried out. The authors would also like to thank the Malaysian Palm Oil Board for their support, particularly Dr. Abdul Gapor for generously providing TRF and purified tocotrienol isoforms for use in this work.

18. REFERENCES

1. Marshall, E.: Epidemiology. Search for a killer: focus shifts from fat to hormones. *Science* 259, 618-621 (1993)

2. Vogel, V.G., A. Yeomans, & E. Higginbotham: Clinical management of women at increased risk for breast cancer. *Breast Cancer Res Treat* 28, 195-210 (1993)

3. Medina, D.: Mammary tumorigenesis in chemical carcinogen-treated mice. I. Incidence in BALB-c and C57BL mice. *J Natl Cancer Inst* 53, 213-221 (1974)

4. Nagasawa, H., R. Yanai, & H. Taniguchi: Importance of mammary gland DNA synthesis on carcinogen-induced mammary tumorigenesis in rats. *Cancer Res* 36, 2223-2226 (1976)

5. Russo, I.H. & J. Russo: Developmental stage of the rat mammary gland as determinant of its susceptibility to 7, 12-dimethylbenz [a] anthracene. *J Natl Cancer Inst* 61, 1439-1449 (1978)

6. Sylvester, P.W., C.F. Aylsworth, D.A. Van Vugt, & J. Meites: Effects of alterations in early hormonal environment on development and hormone dependency of carcinogen-induced mammary tumors in rats. *Cancer Res* 43, 5342-5346 (1983)

7. Sylvester, P.W., C. Ip, & M.M. Ip: Effects of high dietary fat on the growth and development of ovarian-independent carcinogen-induced mammary tumors in rats. *Cancer Res* 46, 763-769 (1986)

8. Guzman, R.C., R.C. Osborn, S.M. Swanson, R. Sakthivel, S.I. Hwang, S. Miyamoto, & S. Nandi: Incidence of c-Ki-ras activation in N-methyl-N-nitrosourea-induced mammary carcinomas in pituitary-isografted mice. *Cancer Res* 52, 5732-5737 (1992)

9. Imagawa, W., G.K. Bandyopadhyay, M. Garcia, A. Matsuzawa, & S. Nandi: Pregnancy-dependent to ovarian-independent progression in mammary tumors delineated in primary culture: changes in signal transduction, growth factor regulation, and matrix interaction. *Cancer Res* 52, 6531-6538 (1992)

10. Swanson, S.M., R.C. Guzman, G. Collins, P. Tafoya, G. Thordarson, F. Talamantes, & S. Nandi: Refractoriness to mammary carcinogenesis in the parous mouse is reversible by hormonal stimulation induced by pituitary isografts. *Cancer Lett* 90, 171-181 (1995)

11. Swanson, S.M., R.C. Guzman, T. Tsukamoto, T.T. Huang, C.D. Dougherty, & S. Nandi: N-Ethyl-N-nitrosourea induces mammary cancers in the pituitary-isografted mouse which are histologically and genotypically distinct from those induced by N-methyl-N-nitrosourea. *Cancer Lett* 102, 159-165 (1996)

12. Nandi, S., R.C. Guzman, & J. Yang: Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. *Proc Natl Acad Sci U S A* 92, 3650-3657 (1995)

13. Goh, S.H., N.F. Hew, A.W. Norhanom, & M. Yadav: Inhibition of tumour promotion by various palm-oil tocotrienols. *Int J Cancer* 57, 529-531 (1994)

14. Rogers, A.E. & S.Y. Lee: Chemically-induced mammary gland tumors in rats: modulation by dietary fat. *Prog Clin Biol Res* 222, 255-282 (1986)

15. Sundram, K., H.T. Khor, & A.S. Ong: Effect of dietary palm oil and its fractions on rat plasma and high density lipoprotein lipids. *Lipids* 25, 187-193 (1990)

16. Sylvester, P.W., M. Russell, M.M. Ip, & C. Ip: Comparative effects of different animal and vegetable fats fed before and during carcinogen administration on mammary tumorigenesis, sexual maturation, and endocrine function in rats. *Cancer Res* 46, 757-762 (1986)

17. Nakayama, M., H.R. Ju, M. Sugano, N. Hirose, T. Ueki, F. Doi, & A.R. Eynard: Effect of dietary fat and cholesterol on dimethylbenz [a]-anthracene- induced mammary tumorigenesis in Sprague-Dawley rats. *Anticancer Res* 13, 691-698 (1993)

18. Cottrell, R.C.: Introduction: nutritional aspects of palm oil. *Am J Clin Nutr* 53, 989S-1009S (1991)

19. Packer, L.: Protective role of vitamin E in biological systems. *Am J Clin Nutr* 53, 1050S-1055S (1991)

20. Elson, C.E.: Tropical oils: nutritional and scientific issues. *Crit Rev Food Sci Nutr* 31, 79-102 (1992)

21. Kimmick, G.G., R.A. Bell, & R.M. Bostick: Vitamin E and breast cancer: a review. *Nutr Cancer* 27, 109-117 (1997)

22. HT Khor, & R Raajeswari: Red palm oil, vitamin A, and the antioxidant enzymes. In: Micronutrients and Health: Molecular Biological Mechanisms. Eds: Nesaretnam K, & Packer L, AOCS Press: Champaign, IL. (2001).

23. Sundram, K., H.T. Khor, A.S. Ong, & R. Pathmanathan: Effect of dietary palm oils on mammary carcinogenesis in female rats induced by 7, 12-dimethylbenz (a) anthracene. *Cancer Res* 49, 6, 1447-51 (1989)

24. McIntyre, B.S., K.P. Briski, A. Gapor, & P.W. Sylvester: Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. *Proc Soc Exp Biol Med* 224, 292-301 (2000)

25. McIntyre, B.S., K.P. Briski, M.A. Tirmenstein, M.W. Fariss, A. Gapor, & P.W. Sylvester: Antiproliferative and apoptotic effects of tocopherols and tocotrienols on normal mouse mammary epithelial cells. *Lipids* 35, 171-180 (2000)

26. Nesaretnam, K., H.T. Khor, J. Ganeson, Y.H. Chong, K. Sundram, & A. Gapor: The effects of vitamin E tocotrienols from palm oil on chemically induced mammary carcingenesis in female rats. *Nutr. Res* 12, 879-892 (1992)

27. Suzuki YJ, M. Tsuchiya, S.R. Wassall, Y.M. Choo, G. Govil, V.E. Kagan, & L. Packer: Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. *Biochemistry* 32, 10692-10699 (1993)

28. Serbinova, E., V. Kagan, D. Han, & L. Packer: Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. *Free Radic Biol Med* 10, 263-275 (1991)

29. Serbinova, E.A. & L. Packer: Antioxidant properties of alpha-tocopherol and alpha-tocotrienol. *Methods Enzymol* 234, 354-366 (1994)

30. Gould, M.N., J.D. Haag, W.S. Kennan, M.A. Tanner, & C.E. Elson: A comparison of tocopherol and tocotrienol for the chemoprevention of chemically induced rat mammary tumors. *Am J Clin Nutr* 53, 1068S-1070S (1991)

31. Zurinah, W., W. Ngah, Z. Jarien, M.M. San, A. Marzuki, G.D. Top, N.A. Shamaan, & K.A. Kadir: Effect of tocotrienols on hepatocarcinogenesis induced by 2-acetylaminofluorene in rats. *Am. J. Clin. Nutr*. 53, 1076S-1081S (1991)

32. Hosomi, A., M. Arita, Y. Sato, C. Kiyose, T. Ueda, O. Igarashi, H. Arai, & K. Inoue: Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett* 409, 105-108, (1997)

33. Hayes, K.C., A. Pronczuk, & J.S. Liang: Differences in the plasma transport and tissue concentrations of tocopherols and tocotrienols: observations in humans and hamsters. *Proc Soc Exp Biol Med* 202, 353-359 (1993)

34. Marzuki, A., F. Arshad, T.A. Razak, & K. Jaarin: Influence of dietary fat on plasma lipid profiles of Malaysian adolescents. *Am J Clin Nutr* 53, 1010S-1014S (1991)

35. Lanza E, M.R. Forman, E.J. Johnson, R.A. Muesing, B.I. Graubard, & G.R. Beecher: a-tocopherol concentrations in plasma but not in lipoproteins fluctuate during the menstrual cycle in healthy premenopausal women. *J Nutr* 128, 1150-1155 (1998)

36. Tan, D.T., H.T. Khor, W.H. Low, A. Ali, & A. Gapor: Effect of a palm-oil-vitamin E concentrate on the serum and lipoprotein lipids in humans. *Am J Clin Nutr* 53, 1027S-1030S (1991)

37. Ikeda, I., Y. Imasato, E. Sasaki, & M. Sugano: Lymphatic transport of alpha-, gamma- and delta-tocotrienols and alpha- tocopherol in rats. *Int J Vitam Nutr Res* 66, 217-221 (1996)

38. Herrera, E. & C. Barbas.: Vittamin E: action, metabolism and perspectives. *J Physiol Biochem* 57, 43-56 (2001)

39. Yap, S.P., K.H. Yuen, & J.W. Wong: Pharmacokinetics and bioavailablity of alpha-, gamma-, and delta-tocotrienols under different food status. *J Pharmaceut Pharmacol* 56, 67-71 (2001)

40. Sylvester, P.W., B.S. McIntyre, A. Gapor, & K.P. Briski: Vitamin E inhibition of normal mammary epithelial cell growth is associated with a reduction in protein kinase C (alpha) activation. *Cell Prolif* 34, 347-357 (2001)

41. Sylvester, P.W., A. Nachnani, S. Shah, & K.P. Briski: Role of GTP-binding proteins in reversing the antiproliferative effects of tocotrienols in preneoplastic mammary epithelial cells. *Asia Pacific J Clin Nutr* 11, S452-S459 (2002)

42. Sylvester, P.W. & A. Theriault: Role of tocotrienols in the prevention of cardiovascular disease and breast cancer. *Curr Top Nutraceutical Res* 1, 121-136 (2003)

43. Shah, S., A. Gapor, & P.W. Sylvester: Role of caspase-8 activation in mediating vitamin E-induced apoptosis in murine mammary cancer cells. *Nutr Cancer* 45, 236-246 (2003)

44. Ullrich, A. & J. Schlessinger: Signal transduction by receptors with tyrosine kinase activity. *Cell* 61, 2, 203-212 (1990)

45. Imagawa, W., G.K. Bandyopadhyay, & S. Nandi: Regulation of mammary epithelial cell growth in mice and rats. *Endocr Rev* 11, 494-523 (1990)

46. Sylvester, P.W., H.P. Birkenfeld, H.L. Hosick, & K.P. Briski: Fatty acid modulation of epidermal growth factor-induced mouse mammary epithelial cell proliferation *in vitro*. *Exp Cell Res* 214, 145-153 (1994)

47. Birkenfeld, H.P., B.S. McIntyre, K.P. Briski, & P.W. Sylvester: Protein kinase C isoenzyme expression in normal mouse mammary epithelial cells grown in primary culture. *Proc Soc Exp Biol Med* 213, 65-70 (1996)

48. Clement, S., A. Tasinato, D. Boscoboinik, & A. Azzi: The effect of alpha-tocopherol on the synthesis, phosphorylation and activity of protein kinase C in smooth muscle cells after phorbol 12-myristate 13-acetate down-regulation. *Eur J Biochem* 246, 745-749 (1997)

49. Ricciarelli, R., A. Tasinato, S. Clement, N.K. Ozer, D. Boscoboinik, & A. Azzi: alpha-Tocopherol specifically inactivates cellular protein kinase C alpha by changing its phosphorylation state. *Biochem J* 334, 243-249 (1998)

50. Bandyopadhyay, G.K., W. Imagawa, & S. Nandi: Role of GTP-binding proteins in the polyunsaturated fatty acid stimulated proliferation of mouse mammary epithelial cells. *Prostaglandins Leukot Essent Fatty Acids* 52, 151-158 (1995)

51. Xing, C. & W. Imagawa: Altered MAP kinase (ERK1, 2) regulation in primary cultures of mammary tumor cells: elevated basal activity and sustained response to EGF. *Carcinogenesis* 20, 1201-1208 (1999)

52. Richards, J.S.: New signaling pathways for hormones and cyclic adenosine 3', 5'-monophosphate action in endocrine cells. *Mol Endocrinol* 15, 209-218 (2001)

53. Rommel, C., B.A. Clarke, S. Zimmermann, L. Nunez, R. Rossman, K. Reid, K. Moelling, G.D. Yancopoulos, & D.J. Glass: Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt. *Science* 286, 1738-1741 (1999)

54. Zimmermann, S. & K. Moelling: Phosphorylation and regulation of Raf by Akt (protein kinase B). *Science* 286, 1741-1744 (1999)

55. Shah, S.J. & P.W. Sylvester: Tocotrienol-induced caspase-8 activation occurs independently of death receptor apoptotic signaling in malignant mammary epithelial cells. *Exp Biol Med* 229, 745-755 (2004)

56. Baldwin, A.S., Jr.: The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 14, 649-683 (1996)

57. Biswas, D.K., A. Cruz, N. Pettit, G.L. Mutter, & A.B. Pardee: A therapeutic target for hormone-independent estrogen receptor-positive breast cancers. *Mol Med* 7, 59-67 (2001)

58. Biswas, D.K., S.C. Dai, A. Cruz, B. Weiser, E. Graner, & A.B. Pardee: The nuclear factor kappa B (NF-kappa B): a potential therapeutic target for estrogen receptor negative breast cancers. *Proc Natl Acad Sci U SA* 98, 10386-10391 (2001)

59. Sliva, D., M.T. Rizzo, & D. English: Phosphatidylinositol 3-kinase and NF-kappaB regulate motility of invasive MDA-MB-231 human breast cancer cells by the secretion of urokinase-type plasminogen activator. *J Biol Chem* 277, 3150-3157 (2002)

60. Khwaja, A.: Akt is more than just a Bad kinase. *Nature* 401, 33-34 (1999)

61. Kumar, R., R.K. Vadlamudi, & L. Adam: Apoptosis in mammary gland and cancer. *Endocr Relat Cancer* 7, 257-269 (2000)

62. Kerr, J.F., A.H. Wyllie, & A.R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26, 239-257 (1972)

63. Earnshaw, W.C., L.M. Martins, & S.H. Kaufmann: Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 68, 383-424 (1999)

64. Slee, E.A., C. Adrain, & S.J. Martin: Serial killers: ordering caspase activation events in apoptosis. *Cell Death Differ* 6, 1067-1074 (1999)

65. Bratton, S.B., M. MacFarlane, K. Cain, & G.M. Cohen: Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. *Exp Cell Res* 256, 27-33 (2000)

66. Sun, X.M., M. MacFarlane, J. Zhuang, B.B. Wolf, D.R. Green, & G.M. Cohen: Distinct caspase cascades are initiated in receptor-mediated and chemical-induced apoptosis. *J Biol Chem* 274, 5053-5060 (1999)

67. Schulze-Osthoff, K., D. Ferrari, M. Los, S. Wesselborg, & M.E. Peter: Apoptosis signaling by death receptors. *Eur J Biochem* 254, 439-459 (1998)

68. Panka, D.J., T. Mano, T. Suhara, K. Walsh, & J.W. Mier: Phosphatidylinositol 3-kinase/Akt activity regulates c-FLIP expression in tumor cells. *J Biol Chem* 276, 6893-6896 (2001)

69. Suhara, T., T. Mano, B.E. Oliverira, & K. Walsh: Phosphatidylinositol 3-kinase/Akt signaling coontrols endothelial cell sensitivity to Fas-mediated apoptosis via regulation of FLICE-inhibitory protein (FLIP). *Circ Res* 89, 13-19 (2001)

70. Varadhachary, A.S., M. Edidin, A.M. Hanlon, M.E. Peter, P.H. Krammer, & P. Salgame: Phosphatidylinositol 3'-kinase blocks CD95 aggregation and caspase-8 cleavage at the death-inducing signaling complex by modulating lateral diffusion of CD95. *J Immunol* 166, 6564-6569 (2001)

71. Green, D.R. & J.C. Reed: Mitochondria and apoptosis. *Science* 281, 1309-1312 (1998)

72. Li, H., H. Zhu, C.J. Xu, & J. Yuan: Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94, 491-501 (1998)

73. Antonsson, B. & J.D. Martinou: The Bcl-2 protein family. *Exp Cell Res* 256, 50-57 (2000)

74. Desagher, S., A. Osen-Sand, A. Nichols, R. Eskes, S. Montessuit, S. Lauper, K. Maundrell, B. Antonsson, & J.C. Martinou: Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. *J Cell Biol* 144, 891-901 (1999)

75. Chen, Q., B. Gong, & A. Almasan: Distinct stages of cytochrome c release from mitochondria: evidence for a feedback amplification loop linking caspase activation to mitochondrial dysfunction in genotoxic stress induced apoptosis. *Cell Death Differ* 7, 227-233 (2000)

76. Kirsch, D.G., A. Doseff, B.N. Chau, D.S. Lim, N.C. de Souza-Pinto, R. Hansford, M.B. Kastan, Y.A. Lazebnik, & J.M. Hardwick: Caspase-3-dependent cleavage of Bcl-2 promotes release of cytochrome c. *J Biol Chem* 274, 21155-21161 (1999)

77. Sylvester, P.W., & S. Shah.: Antioxidants in dietary oils: their potential role in breast cancer prevention. *Malaysian J Nutr* 8, 1-11 (2002)

78. Walczak, H., & P.H. Krammer: The CD95 (APO-1/Fas) and the TRAIL (APO-2L) apoptosis systems. *Exp Cell Res* 256, 58-66 (2000)

79. Yu, W., M. Simmons-Menchaca, A. Gapor, B.G. Sanders, & K. Kline: Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. *Nutr Cancer* 33, 26-32 (1999)

80. Yu, W., K. Israel, Q.Y. Liao, C.M. Aldaz, B.G. Sanders, & K. Kline: Vitamin E succinate (VES) induces Fas sensitivity in human breast cancer cells: role for Mr 43,000 Fas in VES-triggered apoptosis. *Cancer Res* 59, 953-961 (1999)

81. Reed, J.C.: Dysregulation of apoptosis in cancer. *J Clin Oncol* 17, 2941-2953 (1999)

82. Hughes, S.J., Y. Nambu, O.S. Soldes, D. Hamstra, A. Rehemtulla, M.D. Iannettoni, M.B. Orringer, & D.G. Beer: Fas/APO-1 (CD95) is not translocated to the cell membrane in esophageal adenocarcinoma. *Cancer Res* 57, 5571-5578 (1997)

83. Yeh, J.H., S.C. Hsu, S.H. Han, & M.Z. Lai: Mitogen-activated protein kinase kinase antagonized fas-associated death domain protein-mediated apoptosis by induced FLICE-inhibitory protein expression. *J Exp Med* 188, 1795-1802 (1998)

84. Xiao, C.W., E. Asselin, & B.K. Tsang: Nuclear factor kappaB-mediated induction of Flice-like inhibitory protein prevents tumor necrosis factor alpha-induced apoptosis in rat granulosa cells. *Biol Reprod* 67, 436-441 (2002)

85. Neve, R.M., T. Holbro, & N.E. Hynes: Distinct roles for phosphoinositide 3-kinase, mitogen-activated protein kinase and p38 MAPK in mediating cell cycle progression of breast cancer cells. *Oncogene* 21, 4567-4576 (2002)

86. Hermanto, U., C.S. Zong, & L.H. Wang: ErbB2-overexpressing human mammary carcinoma cells display an increased requirement for the phosphatidylinositol 3-kinase signaling pathway in anchorage-independent growth. *Oncogene* 20, 7551-7562 (2001)

87. Vanhaesebroeck, B. & M.D. Waterfield: Signaling by distinct classes of phosphoinositide 3-kinases. *Exp Cell Res* 253, 239-254 (1999)

88. Varadhachary, A.S., M.E. Peter, S.N. Perdow, P.H. Krammer, & P. Salgame: Selective up-regulation of phosphatidylinositol 3'-kinase activity in Th2 cells inhibits caspase-8 cleavage at the death-inducing complex: a mechanism for Th2 resistance from Fas-mediated apoptosis. *J Immunol* 163, 4772-4779 (1999)

89. Kandel, E.S. & N. Hay: The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. *Exp Cell Res* 253, 210-229 (1999)

90. Downward, J.: Mechanisms and consequences of activation of protein kinase B/Akt. *Curr Opin Cell Biol* 10, 262-267 (1998)

91. Hu, J.S., G.A. Hastings, S. Cherry, R. Gentz, S. Ruben, & T.A. Coleman: A novel regulatory function of proteolytically cleaved VEGF-2 for vascular endothelial and smooth muscle cells. FASEB J 11, 498-504 (1997)

92. Tschopp, J., M. Irmler, & M. Thome: Inhibition of Fas death signals by FLIPs. *Curr Opin Immunol* 10, 552-558 (1998)

93. Rasper, D.M., J.P. Vaillancourt, S. Hadano, V.M. Houtzager, I. Seiden, S.L. Keen, P. Tawa, S. Xanthoudakis, J. Nasir, D. Martindale, B.F. Koop, E.P. Peterson, N.A. Thornberry, J. Huang, D.P. MacPherson, S.C. Black, F. Hornung, M.J. Lenardo, M.R. Hayden, S. Roy, & D.W. Nicholson: Cell death attenuation by 'Usurpin', a mammalian DED-caspase homologue that precludes caspase-8 recruitment and activation by the CD-95 (Fas, APO-1) receptor complex. *Cell Death Differ* 5, 271-288 (1998)

94. Scaffidi, C., I. Schmitz, P.H. Krammer, & M.E. Peter: The role of c-FLIP in modulation of CD95-induced apoptosis. *J Biol Chem* 274, 1541-1588 (1999)

95. Shain, K.H., T.H. Landowski, & W.S. Dalton: Adhesion-mediated intracellular redistribution of c-Fas-associated death domain-like IL-1-converting enzyme-like inhibitory protein-long confers resistance to CD95-induced apoptosis in hematopoietic cancer cell lines. *J Immunol* 168, 2544-2553 (2002)

96. Tepper, C.G. & M.F. Seldin: Modulation of caspase-8 and FLICE-inhibitory protein expression as a potential mechanism of Epstein-Barr virus tumorigenesis in Burkitt's lymphoma. *Blood* 94, 1727-1737 (1999)

97. Shah, S.J., & P.W. Sylvester: Role of mitochondria and Bcl-2 proteins in mediating apoptotic effects of vitamin E in neoplastic mammary epithelial cells. *Proc Am Assoc Cancer Res* abstract 5162 (2004)

Key Words: vitamin E, Tocotrienols, Mammary Cancer, Apoptosis, Death Receptors, Mitochondrial Stress, Caspase, PI3K, Akt, and NfkappaB, Review

Send correspondence to: Dr Paul W. Sylvester**,** School of Pharmacy, 700 University Avenue, University of Louisiana at Monroe, Monroe, LA, 71209-0470, Tel: 318-342-1958, Fax: 318-342-1606, E-mail: sylvester@ulm.edu