Review

Vitamin E analogues as anticancer agents: Lessons from studies with α-tocopheryl succinate

Xiu-Fang Wang[1], Lanfeng Dong[1], Yan Zhao[2], Marco Tomasetti[3], Kun Wu[2] Jiri Neuzil[1, 4]

[1]Apoptosis Research Group, School of Medical Science, Griffith University, Southport, Qld, Australia
[2]Department of Nutrition and Food, Harbin Medical University, Harbin, Heilongjiang Province, China
[3]Department of Experimental Pathology, Polytechnic of Marche, Ancona, Italy
[4]Laboratory of Cell Signalling and Apoptosis, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

The new millennium has witnessed considerable decrease in a number of previously fatal pathologies, largely due to the advancement in molecular medicine and modern approaches to treatment. In spite of this success, neoplastic disease remains a serious problem due to several reasons. These include an exceedingly high variability of cancer cells even within the same type of tumour. Cancer cells, albeit of clonal origin, mutate so that they escape established treatments, resulting in the fatal outcome of current therapies. Moreover, there are types of cancer, such as mesotheliomas, that cannot be treated at present. A novel group of clinically interesting anticancer drugs has been a recent focus in the literature that hold substantial promise as selective anticancer drugs. These compounds, epitomised by α-tocopheryl succinate, comprise redox-silent analogues of vitamin E that have been shown to suppress several types of cancer in animal models, including breast, colon and lung cancer as well as mesotheliomas and melanomas, while being nontoxic to normal cells and tissues. It is now proven that the strong anticancer effect of vitamin E analogues stems from their propensity to induce selective apoptosis in malignant cells. The results point to the novel group of vitamin E analogues as promising agents applicable to different types of tumours.

Keywords: Vitamin E analogues / Apoptosis / Mitochondria / Signalling pathways / Cancer treatment

Received: December 29, 2005; revised: March 22, 2006; accepted: March 23, 2006

1 Introduction

Neoplastic disease is a complex pathological situation with multiple facets and with exceeding promiscuity in terms of mutations of the relevant genes, necessary for execution of the antitumour activity of established drugs. The clonal origin of cancer cells and their constant mutations make efficient treatment of malignancies an unrelenting challenge. On one hand, some types of cancer are being curbed, on the other hand, others are on the increase or, even worse, beyond treatment at this stage. Therefore, new strategies and approaches are needed to successfully manage the multitude of neoplasias. These should also encompass one of the most coveted features of anticancer agents: selectivity for malignant cells.

We and others have been studying over the last five or so years a novel group of anticancer agents that befit the above scenario, *i.e.* analogues of vitamin E [1–3]. These intriguing compounds have been shown to be highly efficient against a variety of malignancies, including the fatal mesotheliomas. The most studied member of these drugs, α-tocopheryl succinate (α-TOS), exerts its pro-apoptotic activity by triggering a massive apoptogenic response in a variety of cancer cells of different origin as well as by arresting the cell cycle and inhibiting proliferation of cancer cells by disrupting autocrine signalling pathways. The agent has also been shown to be highly selective for malignant cells, being largely nontoxic to normal cells and tissues. Thus, α-TOS and relative compounds may represent the long sought-after drugs of choice for treatment of multiple malignancies [3].

Correspondence: Professor Jiri Neuzil, Apoptosis Research Group, Heart Foundation Research Centre, School of Medical Science, Griffith University Gold Coast Campus, Southport, Qld, Australia
E-mail: j.neuzil@griffith.edu.au
Fax: +61-7-55528444

Abbreviations: AIF, apoptosis-inducing factor; **IAPs,** inhibitors of apoptosis proteins; **NF-κB,** nuclear factor-κB; **ROS,** reactive oxygen species; **α-TOS,** α-tocopheryl succinate

This review summarises our current knowledge on the mechanism of action of vitamin E analogues, epitomised by α-TOS, in the context of their anticancer activity. The paper focuses on their structure–function relationship and the major pathways that they initiate/deregulate, which then translates into efficient inhibition of cancer. Future perspectives of these intriguing compounds are suggested.

2 Structure-function relationship of vitamin E analogues as inducers of apoptosis in malignant cells

Figure 1 shows the major features of the nonapoptogenic α-tocopherol (α-TOH) and the proapoptotic α-TOS [3]. Moieties III and II are shared in both analogues. The *Hydrophobic Moiety* (III) is responsible for docking the compounds in circulating lipoproteins and in biological membranes while the *Signalling Moiety* (II) modulates certain signalling pathways, such as the protein phosphate 2/protein kinase C pathway [4]. The *Functional Moiety* (I) differs though: while it comprises the redox-active hydroxyl group in α-TOH, it is esterified with a succinyl moiety in case of α-TOS. In the case of the latter, the succinyl moiety within Moiety I makes the analogue redox-silent and endows it with strong apoptogenic activity. It has now been well documented that α-TOS is highly proapoptotic towards malignant cells. In fact, at least 50 types of cancer cell lines tested thus far have shown a high level of apoptosis when challenged with α-TOS [3] except for the osteosarcoma cell line MG63, in which α-TOS causes cell cycle arrest rather than inducing apoptosis [5]. Importantly too, α-TOS is largely nontoxic to normal cells [6] and tissues [7].

The eight members of the VE family (four tocopherols and four tocotrienols) each possess a free phenol hydroxyl group in their Functional Moiety. All of these compounds are potent antioxidants, since they are all able to donate a neutral hydrogen atom to an oxidant species. However, majority of these natural vitamin E components are unable to induce apoptosis. For this reason, most research has been directed towards structural variations within the Functional Moiety, with the goal of introducing apoptogenic activity into these compounds.

Except for γ-tocotrienol (γ-T3H) and δ-T3H, other members of the vitamin E family require a modification of the hydroxyl group within the Functional Moiety in order to display apoptogenic activity. Numerous Functional Moiety analogues of α-TOH have been explored, and a number of interesting structure-activity relationships have been demonstrated [8]. The most studied Functional Moiety analogues are tocopheryl monoesters of dicarboxylic acids. These esters all bear an acidic group ($pK_a < 6$) within this moiety [9]. Succinylation of any member of the vitamin E

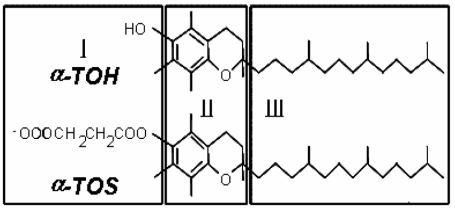

Figure 1. Basic structural features of α-tocopherol and α-tocopheryl succinate. Vitamin E analogues, epitomised here by α-tocopherol and α-tocopheryl succinate, comprise three major moieties, the Functional Moiety (I), the Signalling Moiety (II) and the Hydrophobic Moiety (III).

family of compounds makes them apoptogenic. In the case of γ-T3H, which itself already displays proapoptotic activity, the succinate monoester has even greater activity than the parent compound. The size and nature of the ester substituent also has significant bearing on the observed activity. Both α-TOS and α-tocopheryl maleate (α-TOM) display significant apoptogenic activity. However, this level of activity falls off for the glutarate analogue, and is virtually nondetectable for 2,2-dimethyl- and 3,3-dimethylglutaryl monoesters. Decreased water solubility or ampiphilicity may contribute to the observed trend. Very potent apoptogenic activity is seen for the maleyl monoesters, with α-TOM displaying nearly 20-fold greater potency than α-TOS [8]. Even greater apoptotic efficacy has been observed for the isomer of α-TOM, α-tocopheryl fumarate (α-TOF) (Neuzil *et al.*, unpublished results). The reduced conformational flexibility within dihydrosuccinate esters may contribute to this enhanced activity, but it is not known whether that is the predominant factor. It is possible that the superiority of α-TOF over α-TOM is due to the lower dipole moment of the former, since it will be buried deeper in the lipidic membrane, thereby bringing the chargeable carboxylate closer to the interphase, as suggested for other agents [10].

Esters without acid functionality (*e.g.* α-TOA) or dicarboxylic diesters (*e.g.* α-TOSM) show no apoptogenic activity at all. These observations lead one to believe that a charged group within the Functional Moiety and/or overall amphiphilicity may play a key role in the biological effect of these molecules. Significant activity has also been observed for tocopheryl esters bearing Functional Moiety that is cationic at physiological pH, with α-tocopheryl lysine monoester being among the most striking examples [11].

The Hydrophobic Moiety plays a crucial role in the apoptogenic activity as well. Recent research demonstrates that the lipid tail within the Hydrophobic Moiety is a necessary but not sufficient structural element for the induction of apoptosis. The removal of the lipid tail from the Hydropho-

bic Moiety of active compounds completely wipes out their ability to induce apoptosis. Thus, succinyl esters in which the lipid tail is missing (*e.g.* Trolox succinate) are unable to induce apoptosis [8]. However, nonphysiological concentrations (>1 mM) of analogues of vitamin E lacking the Hydrophobic Moiety, as shown for Trolox, are capable of apoptosis induction, and, notably, sensitize p53-deficient cancer cells to established antitumour drugs by up-regulating the cell cycle check-point protein $p21^{waf1/cip1}$ [12].

In some other cases, however, the nature of the lipid tail in the Hydrophobic Moiety appears to override the need for other key structural elements within the Functional Moiety. For example, even though γ-tocopherol (γ-TOH) is completely nonapoptogenic, γ-tocotrienol (γ-T3h) (possessing a polyunsaturated lipid tail) does display apoptogenic activity [8, 13, 14], similarly as does δ-T3H [15]. Further modification of the Hydrophobic Moiety in γ-tocotrienol also produced interesting results. A synthetic analogue in which the aliphatic side chain of γ-tocotrienol was shortened by one isoprenyl unit proved to be more apoptogenic than γ-tocotrienol itself. The homologue is formed by side-chain modification of γ-T3H by some cells [16]. Interestingly, the nonapoptotic α-T3H is endowed with apoptogenic activity following its esterification [17]. Taken together, these results suggest an interesting interplay between the Functional and Hydrophobic Moieties in establishing apoptogenic activity. The fact that γ-tocotrienol is apoptogenic with just a phenolic group in the Functional Moiety also suggests that multiple pathways may exist through which apoptosis is triggered by members and derivatives of the vitamin E family.

Structural variations within the Signalling Moiety have also been explored. Like the Hydrophobic Moiety, the Signalling Moiety is a necessary but not a sufficient structural element for apoptogenic activity. Elimination of this part from α-TOS has been explored using phytyl succinate and oleyl succinate, compounds that showed absolutely no apoptogenic effect [8]. This result indicates that the proapoptotic activity of tocopheryl dicarboxylic acid monoesters is triggered by more than just simple detergent properties of the lipid ester carboxylate. Apparently, the Signalling Moiety enables the molecule to intervene within key signal transduction pathway(s). Variation of the methyl substitution on the aromatic ring within the tocopherol series also modulates the apoptogenic activity. For example, α-TOS is more potent than the corresponding β-, γ- and δ-TOS analogues. The degree of methyl substitution is also very important in the tocotrienol series [8].

We have recently explored the possibility that modification of the bond between the tocopheryl head group and the side group may also modulate the overall apoptogenic activity of the resulting compound. Thus, we synthesised analogues of vitamin E with an amide bond linking the Signalling and the Functional Moiety and found that they were significantly more apoptogenic than their ester counterparts, with α-tocopherylmaleyl amide (α-TAM) being some 100-fold more efficient in induction of apoptosis in Jurkat cells [17]. A plausible explanation of the superiority of amides over corresponding esters may be their better partitioning into the lipidic phase, whereby more efficiently destabilising biological membranes [17]. It was reported that the amide analogues, such as α-TAM, are very potent inducers of apoptosis for a variety of cancer cell lines, including the erbB2 overexpressing breast cancer cells [18] and neuroblastoma cells [19].

Another possibility of a modification of the basic molecule of α-TOS is replacement of the ester bond with an ether bond. The resulting α-tocopheryloxybutyrate exerts at least a comparable apoptogenic efficacy to that of α-TOS, as shown for leukemia cell lines [20] or even more efficient, as reported for prostate cancer cells [21]. The potential higher efficacy of the ether analogues of α-TOS may be due to a longer half-life *in vivo*, since the ester bond is subject to esterase-dependent hydrolysis while the ether bond is resistant. Another advantage of the ether analogue is that it is not subject to hydrolysis, unlike α-TOS, when ingested orally, and reaches the bloodstream in the form of the original compound, while α-TOS is completely converted into the nonapoptogenic α-TOH unless injected intravenously [22, 23]. On the other hand, the ester analogues may have a lower secondary toxicity than the ether compounds, since the former are gradually converted into α-TOH with a potential secondary beneficial activity [22, 23].

These results clearly show that modifications within the structural moieties of vitamin E analogues modulate their activity and may lead to generation of novel agents with higher apoptotic efficacy and greater selectivity for malignant cells.

3 Mitochondria as mediators of apoptogenic activity of vitamin E analogues

Mitochondria are membrane-enclosed organelles distributed through the cytosol of most eukaryotic cells. Their main function is the conversion of the potential energy of 'food' molecules into ATP. Recent research demonstrated that mitochondria play an important role in the programmed cell death [24], and the role of mitochondria has also been demonstrated for apoptosis induced by vitamin E analogues [3].

Probably, the most compelling evidence for mitochondria as major transmitters of apoptotic signalling induced by vitamin E analogues follows from experiments in which

mtDNA-deficient (ρ^0) cells were found to be resistant to α-TOS when compared to their wild-type and revertant counterparts [18, 25]. It has also been observed that transfection of cancer cells with dominant-negative (DN) caspase-9 or caspase-9 siRNA suppressed apoptosis induced by α-TOS [7, 19]. It has also been reported that cancer cells lacking mtDNA (ρ^0 cells), resistant to apoptosis [26], failed to translocate cytochrome c when challenged with α-TOS, unlike the apoptosis-sensitive parental and revertant cells, and this resistance also included low levels of phosphatidyl serine externalisation and caspase-3 activation [25]. Similar resistance of ρ^0 cells has been found for other inducers of apoptosis, such as tumour necrosis factor-α (TNF-α) [27].

The mitochondrial pro- and antiapoptotic proteins, including bax, bcl-2 and bcl-x_L, are important factors related to mitochondrial apoptotic signalling pathways [28]. Generation of the mitochondrial permeability transition pore has also been suggested in cells exposed to α-TOS [29]. It is likely that this is modulated by a crosstalk between the mitochondrial pro- and antiapoptotic proteins [25, 29]. Overexpression of bax sensitised cells to α-TOS-induced apoptosis [25, 30], whereas overexpression of bcl-2 or bcl-x_L protected them from α-TOS; this was not observed when truncated proteins lacking the mitochondrial-targeting terminus were used [25]. Similarly, down-regulation of bcl-2 by antisense oligodoxynucleotide treatment sensitised cells to the vitamin E analogue [6, 25]. Finally, transfection with a gain-of-function mutant of bcl-2 protected, while a loss-of-function mutant of the protein sensitised cancer cells: in these mutant versions of bcl-2, serine 70 was replaced with glutamine and alanine, respectively. This observation can be explained by PKC-dependent phosphorylation of S70 that plays a role in mitochondrial docking of bcl-2 [31].

Mitochondria are sites of downstream mediators whose relocalisation relays further the upstream proapoptotic signals. The downstream events following mitochondrial destabilisation in apoptosis induced by VE analogues comprise mobilisation of apoptotic mediators, which includes cytochrome c, the apoptosis-inducing factor (AIF) and Smac/Diablo. Cytochrome c, upon cytosolic translocation, forms a ternary complex with Apaf-1 and procaspase-9, leading to autoactivation of the initiator caspase-9 with ensuing activation of the effector caspase-3, -6 or -7. At this stage, the cell enters the 'point of no return', *i. e.* the irreversible phase of the apoptotic pathway [6, 25, 29]. This pathway is critically important in apoptosis induced by α-TOS [3]. Smac/Diablo is an important agonist of the caspase-dependent apoptotic signalling, since it antagonises the caspase-inhibitory members of the family of inhibitors of apoptosis proteins (IAPs), including c-IAP1, c-IAP2 and X-IAP [32, 33]. The expression of IAPs is under control of the transcriptional factor nuclear factor-κB (NF-κB), whose activity is inhibited by α-TOS [34–36]. Thus, cytosolic translocation of Smac/Diablo may promote inhibition of the survival pathways in apoptosis induced by α-TOS, which may maximise the apoptogenic potential in resistant cells [18, 25].

Another mitochondrial protein amplifying apoptosis in cells exposed to VE analogues is AIF [25] that translocates directly into the nuclei, thereby bypassing the caspase activation cascade [37]. Thus, AIF, upon translocation to the nucleus, triggers cleavage of chromatin in a caspase-independent manner [38]. AIF can thus avoid mutations in the caspase-dependent signalling that can render the cancer cell resistant to treatment and may mediate α-TOS-induced apoptosis in cells resistant to conventional anticancer drugs that rely solely on caspase activation.

While the events in apoptosis induced by vitamin E analogues downstream of mitochondrial are relatively understood, much less is known about the initial triggers. There is evidence, however, that treatment of cells with α-TOS causes generation of reactive oxygen species (ROS) [18, 19, 25, 39–43]. The major form of ROS generated by cells in response to α-TOS appears to be superoxide, since addition of superoxide dismutase removed the radicals and also inhibited apoptosis [18, 40]. Moreover, the site of superoxide generation as well as the target of the ROS are very likely mitochondria, as suggested by experiments, in which mitochondrially targeted coenzyme Q [44] suppressed radical generation and inhibited apoptosis induced by α-TOS in cancer cells [18, 25, 45]. Also, it has been reported that α-TOS-induced apoptosis was more pronounced in cancer cells with low efficacy of the antioxidant machinery [41]. Generation of radicals appears to be a relatively early event in cells as a response to vitamin E analogues, since we observed substantial accumulation of ROS in Jurkat cells after 1 h of α-TOS challenge.

The earliest effect observed upon exposure of cells to α-TOS is activation of sphingomyelinase (SMase), an enzyme that converts sphingomyelin, which is a relatively rare lipid constituent of the plasma membrane, to the apoptogenic ceramide [46]. Treatment of Jurkat cells resulted in activation of SMase within 15–30 min and was not suppressed by a caspase inhibitor, suggesting a caspase-independent, probably direct target of the vitamin E analogue [25]. It is possible that the activation is due to a change in the plasma membrane fluidity upon incorporation of the lipophilic α-TOS, consistent with a recently suggested mechanism [47]. Generation of the lipid second messenger ceramide in cancer cells as a very early response to α-TOS may also provide an explanation for the activation of protein phosphatase 2A (PP2A) and the ensuing hypophosphorylation of protein kinase C-α in cells exposed to α-TOS, since the drug does not directly target PP2A [6]. This hypothesis is in agree-

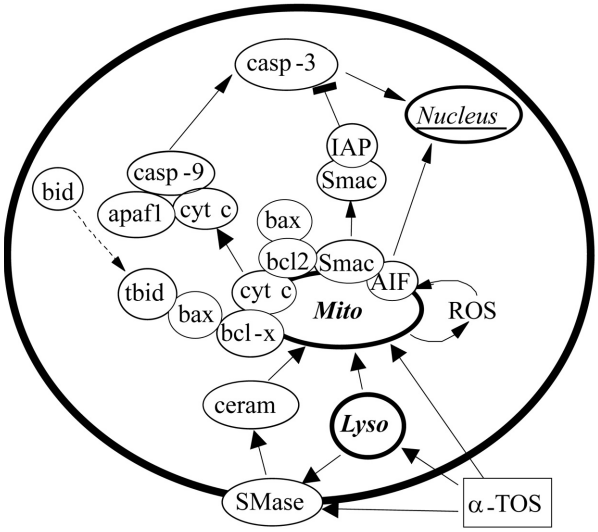

Figure 2. Possible upstream and downstream intrinsic apoptotic pathways initiated by vitamin E analogues, based largely on experiments with Jurkat cells (adapted from [25]). α-TOS translocates to the cell, activates SMase, giving rise to the formation of the lipid second message ceramide, possibly causing also destabilisation of lysosomes. These events converge on destabilisation of the mitochondrial membrane, which is amplified by ROS generation during this process. Mitochondrial membrane destabilisation, likely promoted by leakage by lysosomal proteases, leads to cytosolic relocalisation of proapoptotic factors (such as cytochrome c, Smac/Diablo or AIF) that can be regulated by the Bcl-2 family of proteins (including the antiapoptotic proteins Bcl-2, Bcl-x_L or Mcl-1, which can be compromised by the proapoptotic Bax, probably mobilised to mitochondria after cleavage of Bid to its proapoptotic form tBid). Cytochrome c, Apaf-1 and procaspase-9 form apoptosome, a ternary complex resulting in activation of the initiator caspase-9 that, in turn, activates the effector caspases. Smac/Diablo may amplify this process by suppressing the caspase-inhibitory activity of IAP family proteins, while AIF transmits the mitochondrial destabilisation directly to nuclear apoptotic events, bypassing the caspase cascade.

ment with the previous finding that long-chain ceramides are activators of PP2A [48].

An intriguing aspect of apoptosis induced by vitamin E analogues is that it may also involve the role of lysosomes [49]. We have shown that α-TOS caused early lysosomal destabilisation [50] and that cells made deficient in an important lysosomal protein, cathepsin D, were relatively resistant to the vitamin E analogue [9]. Although experiments with fibroblasts from mucolipidosis II patients, featuring impaired lysosomal sorting of proteins, showed resistance to apoptosis induced by chemotherapeutic agents [51], it is highly probable that lysosomal destabilisation is upstream of mitochondria in apoptosis signalling triggered by vitamin E analogues and amplifies the major, mitochondrial pathway rather than bringing the cells to the commitment phase in a mitochondria-independent manner [3] (Fig. 2).

While mitochondria are essential for initiation of apoptotic signalling by α-TOS, other analogues may exert some unrelated activity. This is particularly relevant to compounds like γ-T3H and δ-T3H, whose antiproliferative/proapoptotic effect may be mediated by mitochondria-dependent [52] as well as independent events [15, 53, 54].

4 Deregulation of signalling pathways by vitamin E analogues

Although mitochondria are central to apoptosis induction by vitamin E analogues in cancer cells, presumably due to interference of compounds like α-TOS with ubiquinone binding on the mitochondrial complex II (Dong *et al.*, submitted), there are other pathways modulated by these agents that may run parallel to the major, mitochondrial apoptotic signalling or may amplify it [25, 29, 30, 50]. The downstream signals originating from mitochondria are initiated by cytosolic translocation of the mediators cytochrome c [18, 25, 30], AIF [25] or Smac/Diablo [18, 25], all of which may relocate as a response to exposure of cells to vitamin E analogues, as shown primarily for α-TOS. These pathways result in either caspase-dependent (cytochrome c) and caspase-independent apoptosis (AIF) or in secondary modulation of other signalling pathways (Smac/Diablo). The cytochrome c- and AIF-dependent pathways were discussed in the previous chapter, the role of Smac/Diablo is covered below.

Of the signalling pathways modulated by vitamin E analogues, some are implicated in high levels of malignancy and resistance of cancers to established treatments. One of them, encountered in breast cancer, stems from overexpression of erbB2, a receptor tyrosine kinase proto-oncogene. ErbB2 is a member of the epithelial growth factor receptor superfamily and a product of the *c-neu* gene [55, 56]. This tyrosine kinase-linked transmembrane protein is overexpressed in > 30% of primary breast cancers. The major complication associated with erbB2 overexpression is linked to spontaneous activation of Akt *via* the phosphatidylinositol 3-kinase pathway [57, 58]. Akt is a serine/threonine kinase that promotes cellular survival [59]. Once activated, Akt exerts antiapoptotic effects through phosphorylation of proteins, such as Bad [60] or caspase-9 [61]. Moreover, Akt causes activation of the transcriptional factor NF-κB [62] that controls expression of prosurvival genes, such as members of the IAPs family [63]. In most of the nontransformed cells, NF-κB complexes (a heterotrimer composed of p50 and p65 subunits bound to an inhibitor, IκB) are largely cytoplasmic. Activators of NF-κB cause fast phosphorylation and ubiquitination of IκB proteins, which are subsequently degraded. This liberates p65, allowing it to transfer to the nucleus, where it triggers transcription of specific

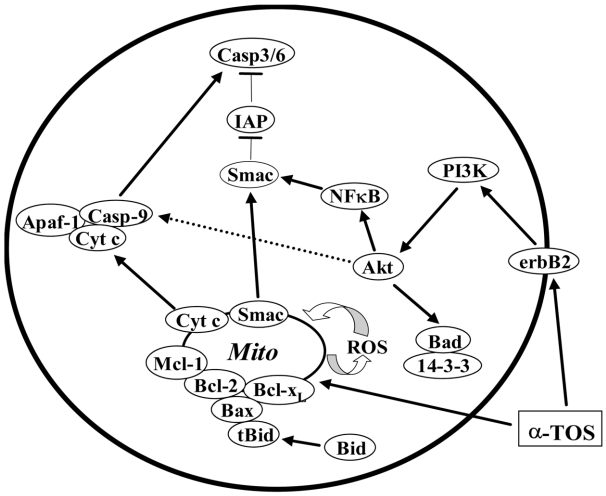

Figure 3. Signalling pathways involved in α-TOS-induced apoptosis in malignant cells overexpressing the erbB2 receptor tyrosine kinase suggest inhibition of the prosurvival pathways (adapted from [18]). α-TOS induces dissipation of the mitochondrial inner membrane potential, and release of mitochondrial apoptotic proteins, such as AIF, Smac/Diablo, and cytochrome c. In many cases, relocalisation of cytochrome c leads to the formation of the apoptosome, a complex consisting of cytochrome c, Apaf-1 and procaspase 9, with ensuing activation of caspase-9, followed by activation of caspase-3. Smac/Diablo may amplify this process by suppressing the antiapoptotic activity of the IAP family proteins. Activated erbB2 can activate Akt *via* PI3K pathway. Once activated, Akt phosphorylates Bad, caspase-9, and inhibits apoptosis. Moreover, Akt causes activation of NF-κB, which then moves to the nucleus and induces expression of the IAP family proteins and Akt itself. α-TOS induces apoptosis regardless of erbB2 by relocalisation of Smac from mitochondria to the cytosol, where Smac binds to IAP so that caspases are ‚liberated' to execute its apoptosis function.

genes, especially those genes involved in immune activation, cell proliferation, apoptosis and cell survival. Thus, inhibition of NF-κB activation may be critical for 'opening' apoptotic pathways in resistant cells.

We showed that vitamin E analogues induced apoptosis at comparable levels in both erbB2-low and -high mouse and human breast cancer cells, regardless of their erbB2 statues. One plausible mechanism is that these agents induce relocalisation of Smac/Diablo from mitochondria to the cytosol [18], where Smac/Diablo binds to IAPs so that caspase-3 is 'free' to execute its apoptosis function [32] (Fig. 3). In another report, it has been shown that α-tocopheryloxybutyric acid, a compound analogous to α-TOS, induced apoptosis in the erbB2 overexpressing human breast cancer cells MDA-MB-453 by simultaneously inhibiting activation of erbB2 (its phosphorylation) and activating the p38 MAP kinase [64]. Several other papers showed modulation of the MAP kinase pathway by vitamin E analogues as a way by which the agents induce apoptosis. Interestingly, Kline's group [65] reported that extracellular signal-regulated kinases (ERKs) and the c-Jun NH_2-terminal kinase (JNK), but not the p38 MAP kinase, were involved in α-TOS-induced apoptosis in the human breast cancer cells MDA-MB-435 cells, and this activated the downstream transcription factors c-Jun and ATF-2. It is possible that this pathway is targeted by α-TOS in the erbB2-low MDA-MB-435 cells while the erbB2-high MDA-MB-453 cells activate their apoptotic machinery by concerted deregulation of the erbB2/Akt and p38 pathways when challenged with vitamin E analogues. Activation of JNK by α-TOS has also been shown for gastric cancer cells [66], and it may amplify the mitochondrial apoptosis signalling pathway, as shown for prostate cancer cells [67].

One of the intriguing targets of vitamin E analogues is the prosurvival transcription factor NF-κB (see above). Inhibition of activation of NF-κB by α-TOS was first documented in the context of cardiovascular disease [34]. One possibility is that the vitamin E analogues trigger apoptosis resulting in activation of caspase-3 that cleaves the obligatory NF-κB subunit p65, rendering it inactive [68]. We have shown that α-TOS initiates a 'subapoptotic' phenotype, under which cells activate their effector caspase but do not enter the commitment phase [35], probably because this requires efficient activation of cyclin-dependent kinases (CDK) [69]. Regardless of the precise mechanism, inhibition of NF-κB activation by vitamin E analogues is antisurvival, *i. e*. proapoptotic, and can also be implicated in adjuvant cancer therapy, as shown for the T lymphoma Jurkat cells, whose treatment with α-TOS sensitised them to TRAIL-dependent killing [36].

Several reports also implicated inhibition of the cell cycle progression as a means by which vitamin E analogues may induce apoptosis or inhibit proliferation of cancer cells and/or sensitise them to other anticancer drugs. Ni *et al.* [70] showed that α-TOS inhibits proliferation of prostate cancer cells by down-regulating expression of several critical cyclins and the cognate CDK, resulting in hypophosphorylation of the Rb protein and a G1/S arrest. Cell cycle arrest and apoptosis were also induced by α-TOS in osteosarcoma cells *via* activation of p53 and reduced expression of the transcription factor E2F1, critical for the G1/S transition [71]. Exposure of osteosarcoma cells to α-TOS also promoted a prolonged arrest at the S/G2 border, sensitising the cells to methotrexate-induced apoptosis [5]. These findings can be reconciled with an earlier report in which α-TOS suppressed proliferation of breast cancer cells by inhibiting the E2F1-dependent transactivation *via* increased binding of cyclin A [72]. Apoptosis induction and inhibition of proliferation by α-TOS have also been shown for malignant mesothelioma cells [73], the latter paradigm being due to selective disruption of the FGF-FGFR autocrine signalling

loop, most likely affected by modulation in the E2F1 and egr-1 transactivation potential [43, 74].

Thus, there is ample evidence that vitamin E analogues affect a variety of signalling pathways that either directly trigger apoptosis or amplify/complement other apoptotic signalling pathways, such as those involving mitochondrial destabilisation.

5 Anticancer activity of vitamin E analogues

Great interest has been focused on the potential use of vitamin E analogues as anticancer drugs and adjuvants in the past decades. This is rather logical, since vitamin E analogues, redox-active micronutrients, are consumed in the regular diet and their doses can be increased by food fortification. Importantly too, they are of general benefit and, as a rule, do not exert secondary deleterious effects.

α-TOH exhibits the highest vitamin E bioactivity among the eight forms of vitamin E, as shown by the rat fetal resorption assay. α-TOH is also the form of vitamin E present at the highest concentration in serum and in dietary supplements. Numerous attempts have been made to find out whether dietary vitamin E has antitumorigenic activity. Although the best understood function of vitamin E is its antioxidant propensity, cell culture, animal and epidemiological studies show that certain vitamin E compounds do exhibit antitumour properties. In the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention trial, α-TOH supplements lowered the incidence and mortality of prostate cancer in male Finnish smokers [75], but had no significant effects on other types of tumours [76, 77]. On the other hand, the epidemiological evidence supporting a link between α-TOH or other forms of vitamin E and cancer is limited, and intervention studies are scarce. Several completed studies have provided rather divergent results. At present, the SELECT trial is under way [78]. In this trial, the effect of supplementation with vitamin E and selenium on pancreatic cancer is being studied in a large cohort of elderly men from the USA and Canada. There is hope that α-tocopherol together with selenium may suppress prostate cancer. In support of this, a study in transgenic mice indicated that selenium suppresses prostate cancer in an animal model when combined with α-TOH [79]. Interestingly, novel seleno-analogues of esters of vitamin E, such as α-tocopheryl-2-phenylselenyl succinate, showed higher effect than their parental counterparts [80].

It is now clear that structural modifications of the vitamin E molecule change the anticancer activity of the agents, with the recent focus on the proapoptotic effect of the various forms and analogues of vitamin E. α-TOS, the most studied apoptogenic vitamin E analogue, has rationally been the optimal choice. Several experimental studies have clearly pointed to α-TOS as a promising anticancer agent. Many of the established chemotherapeutic drugs (*e.g.* doxorubicin and cisplatin) kill not only tumour cells but also normal cells, resulting in serious side effects. α-TOS, however, shows unique selectivity in killing of tumour cells, while not harming normal cells and tissues [1, 81]. As mentioned above, structural characteristics between α-TOH and α-TOS are similar except for their Functional Moiety. This relatively small change in the molecular structure greatly alters the biological activity. While, α-TOH is a major chain-breaking antioxidant in the lipid phase, α-TOS, a redox-silent analogue, significantly inhibits tumour growth *via* inhibition of tumour cell proliferation, blockage of cell cycle progression, arrest of DNA synthesis as well as by induction of apoptosis and differentiation [3, 6, 30, 50, 82].

Importantly, α-TOS has shown high levels of apoptosis induction in a variety of cancer cell lines, including different origin regarding the species (human, murine and avian) and tissue type (breast, prostate, lung, stomach, ovary, monocyte, colon and even the mesothelium) [2, 6, 7, 30, 43, 65, 66, 82 – 87]. Different malignant cells also show diverse susceptibility to α-TOS. About 50% of apoptosis was induced by α-TOS treatment at 20 μg/mL for 48 h in MDA-MB-435 human breast cancer cells [30]. Neuzil *et al.* [81] demonstrated that apoptotic rate induced by α-TOS at 50 μM for 12 h varied from 30 to 60% in different malignant cells, while α-TOS at 20 μg/mL for 48 h efficiently triggered 90% of SGC-7901 human stomach cancer cells to undergo apoptosis [87]. In summary, α-TOS is a potent apoptosis inducer selective for malignant cells, while leading to less than 5% apoptosis in normal cells.

Data have been published revealing that the nonantioxidant analogues of vitamin E strongly suppress cancer cell growth *in vivo* as well. Helson *et al.* [88] found for the first time that intraperitoneal administration of α-TOS (50 mg/kg body weight) markedly inhibited the growth of human neuroblastoma cells in athymic mice. Studies followed, demonstrating that exposure to α-TOS efficiently reduced the incidence of breast, colon or stomach cancer and melanomas. Intraperitoneal administration of α-TOS (150 mg/kg body weight) lowered the growth of human breast cancer, colon carcinoma and murine melanoma cells [89 – 91]. In addition, α-TOS (100 mg/kg body weight) significantly decreased the growth of human colorectal cancer xenografts [7] and increased the survival of immunocompromised mice with experimental human peritoneal mesotheliomas [92]. α-TOS (20 mg/kg body weight) also suppressed the number of tumours and their volume in benzo(a)pyrene-induced forestomach carcinoma in female thymus-bearing mice [87]. In addition, it has been reported that α-TOS suppressed colon cancer metastasis into liver and mammary tumour metastasis into lung in nude mice,

Table 1. Effects of vitamin E analogues in selected experimental cancer models

Animal	Inoculated cell line or tumour inducer	Applied dose	Duration of treatment and effect on tumour growth	Reference
Nude mice	MDA-MB-231 human breast cancer cells	150 mg · kg^{-1}day^{-1} in sesame oil	2 weeks; 80–90% tumour dormancy	[89]
Nude mice	B16F10 murine melanoma cells	100 mg · kg^{-1}day^{-1} in sesame oil	2 weeks; 80–90% tumour dormancy, inhibition of liver metastases	[90]
Nude mice	CT-26 colon cancer cells	100 mg · kg^{-1}day^{-1} in 20% DMSO	2 weeks; ~75% inhibition of liver metastases	[91]
Nude mice	B16F10 murine melanoma cells	150 mg · kg^{-1}day^{-1} in sesame oil	2 weeks; ~70% tumour growth inhibition	[93]
Nude mice	HCT116 human colon cancer cells	100 mg/kg in DMSO every third day	10 days; ~75% tumour growth inhibition	[6]
Nude mice	HCT116 human colon cancer cells	50 mg/kg in DMSO every third day plus 20 μg/mouse of hrTRAIL	10 days; ~70% tumour growth inhibition	[7]
Nude mice	Human mesothelioma Ist-Mes2 cells (peritoneal grafts)	100 mg/kg in DMSO every third day	21 weeks; > three-fold increase in survival	[72]
Nude mice	Human mesothelioma Ist-Mes2 cells (s.c. xenografts)	100 mg/kg in DMSO every second day	16 days; > 90% tumour growth inhibition	[43]
Female Kunming mice	Benzo(a)pyrene-induced forestomach tumours	20 mg/kg in corn oil twice per week	4 weeks; ~85% tumour growth inhibition	[87]
Nude mice	MDA-MB-435-FL-GFP human breast cancer cells	36 μg/mouse of α-TEA daily in aerosol	31 days; ~60% tumour growth inhibition	[102]
C57BL/6 mice	3LLD122 murine Lewis lung carcinoma cell line	200 mg/kg α-TOS in ethanol or 200 mg/kg vesiculated α-TOS	20 days; > 70% tumour growth inhibition	[103]
c-Neu mice	Spontaneous breast carcinomas	15 μmol/mouse in corn oil every third day	2 weeks; 20–30% reduction in original tumour size	Dong *et al.*, submitted
Nude mice	MCF-7 human breast cancer cells	100 mg/kg in DMSO every third day	4 weeks; > 30% reduction in original tumour size	Dong *et al.*, submitted

Except for the work by Zhang *et al.* [102] where the vitamin E analogue was administered as aerosol, it was applied by intraperitoneal injection in all other cases listed.

which further strengthens and extends the prospects for α-TOS as an anticancer drug. α-TOS may also exert its anticancer activity *via* down-regulation of vascular endothelial growth factor [89, 93] and sensitisation of resistant cells to other inducers of apoptosis, such as the immunological TNF-related apoptosis-inducing ligand [7, 92].

All the results mentioned above imply that α-TOS is effective only by intraperitoneal injection, not by oral administration, since it is efficiently hydrolysed to α-TOH when administered orally. It is assumed that the ester link in α-TOS is hydrolysed by nonspecific esterases in the intestinal tract before entering the blood stream [94]. Recent progress in this respect has been made by Kline *et al.* [2] who synthesised a novel analogue of vitamin E, 2,5,7,8-tetramethyl-2*R*-(4*R*,8*R*,12-trimethyltridecyl)-chroman-6-yloxyacetic acid (α-TEA). In this compound, malonate is attached to the Functional Moiety of the analogue *via* an ether bond that is not hydrolysed by esterases, endowing the compound with stability superior to its ester counterparts. It has been reported that α-TEA exhibits similar anticancer and apoptogenic properties as does α-TOS in human breast, prostate, colon, lung and endometrium cancer cells and that it is more efficient than α-TOS in apoptosis induction in human ovarian and cervical cancer cells and mouse mammary tumour cells regardless of the mode of administration [84, 95]. This new vitamin E analogue plus α-TAM and α-tocopheryloxybutyrate mentioned above may provide a new strategy to develop and establish the anticancer activity of vitamin E analogues. Some of the recent studies of vitamin E analogues in animal cancer models are presented in Table 1.

A critical issue is the mode by which vitamin E analogues like α-TOS are administered. It is well known that upon ingestion and intestinal uptake, esters of vitamin E are completely cleaved to vitamin E that is then secreted in mesenteric lymph [94, 96]. This can be overcome by using corresponding ether analogues of vitamin E, such as the ether counterpart of α-TOS, α-tocopheryloxybutyric acid [20, 84, 95]. The ester bond within compounds like α-TOS makes oral administration of the agent impossible and it has to be given by intravenous injection. This mode of delivery, as well as intraperitoneal injection, has been used for administering of α-TOS in experimental animals. We followed the pharmacokinetics of α-TOS after a single dose of the agent injected intraperitoneally in a mouse and found that it needed to be injected every 3 days so that its sufficient level

in plasma was maintained [7]. Subsequent studies revealed that upon hepatic uptake, α-TOS is hydrolysed to α-TOH, which is partially resecreted into the circulation [23]. The hepatic processing of vitamin E analogues (including hydrolysis) by nonspecific esterases and sorting by the selective and saturable tocopherol transfer protein, *cf.* [96, 97] make sure that the levels of α-TOS are maintained and that the long-term levels of α-tocopherol do not increase more than two- to three-fold over the baseline.

Intravenous administration of agents like α-TOS are impractical for humans and other modes, such as transdermal delivery may be more useful and, also, safer [98] (Neuzil *et al.*, unpublished data). Another problem that may be encountered is the potential systemic toxicity of some analogues of vitamin E. While α-TOS has been found nontoxic, the much more apoptogenic α-TAM causes severe neurotoxicity when injected into the mouse peritoneum. This can be overcome by changing the formulation of the agent. We have recently found that liposomal encapsulation of α-TAM makes it nontoxic to the animals while still allowing for cancer growth suppression (Neuzil *et al.*, unpublished data).

Taken together, vitamin E analogues with potent apoptogenic activity show efficient anticancer activity *in vitro* and *in vivo* using experimental animal models. However, it is still unclear whether they are also effective in the case of human cancer patients and what the mechanisms of action under such setting would be. More data from experimental, epidemiological and finally clinical studies are necessary for further investigation of these intriguing and highly promising agents, so that they may be established as routine anticancer drugs.

6 Conclusions and future perspectives

Recently, controversy concerning the use of vitamin E supplements has evolved, following a publication by Miller *et al.* [99], in which they suggest, based on a meta-analysis, that large doses of vitamin E worsen the outcome of multiple pathologies. This was soon followed by a paper by Ford *et al.* [100] reporting on a very high percentage of population of the USA (>10%) taking relatively large vitamin E supplements (>400 IU *per* day) on long-term basis. This publication was accompanied by an editorial in which the year 2005 was referred to as 'annus horribilis' for vitamin E [101]. While we agree with the notion that large doses of vitamin E may (in the best case scenario) not be beneficial at all (since the body has a very tight control of circulating levels of vitamin E), we disagree with the term annus horribilis. If 2005 was a 'horrible year', it should refer rather to the companies that supply the market with vitamin E supplements. On the contrary though, the findings that supplementation with 'mega-doses' of vitamin E may be harmful if *good* for normal, *dietary* vitamin E, and gives a strong message that fortification of diet with high levels of vitamin E is not at all beneficial and better be avoided.

Another important message from the above and similar studies is that large doses of free vitamin E do not have, in general terms, an anticancer effect. Thus, we suggest that rather than vitamin E itself, its analogues, such as α-TOS, may prove beneficial as anticancer agents. This premise is based, at this stage, solely on experimental evidence from animal studies, showing strong anticancer efficacy in all types of cancer studied to date. The finding that α-TOS suppresses malignant mesotheliomas in two different experimental settings in nude mice is particularly intriguing. Thus far, very little is known about the effect of vitamin E analogues on cancer in the case of human patients. One of the problems encountered is the process of administration of the agents. Most analogs of vitamin E with anticancer activity are hydrophobic esters that are completely hydrolysed upon intestinal uptake. Thus, other means have to be used to deliver the drugs to the bloodstream. The intraperitoneal or intravenous injection used in animal experiments is not readily applicable to humans or may cause additional burden. A plausible delivery of α-TOS and other analogues of vitamin E with anticancer activity may be achieved by transdermal application of the drug. We are pursuing this option in a limited perspective clinical trial with postchemotherapy malignant mesothelioma patients. The results will be available later this year (2006). Until first data from such tests are obtained, vitamin E analogues remain in the 'folder' of promising anticancer agents that wait being, potentially, developed into drugs efficient against multiple malignancies.

7 References

[1] Prasad, K. N., Kumar, B., Yan, W. D., Hanson, A. J., Cole, W. C., *J. Am. Coll. Nutr.* 2003, *22*, 108 – 117.

[2] Kline, K., Yu, W., Sanders, B. G., *J. Nutr.* 2004, *134*, 3458S – 3462S.

[3] Neuzil, J., Tomasetti, M., Mellick, A. S., Alleva, R., *et al.*, *Curr Cancer Drug Targets* 2004, *4*, 355 – 372.

[4] Neuzil, J., *Br. J. Cancer* 2003, *89*, 1822 – 1826.

[5] Alleva, R., Benassi, M. S., Tomasetti, M., Gellert, N., *et al.*, *Biochem. Biophys. Res. Commun.* 2005, *331*, 1515 – 1521.

[6] Neuzil, J., Weber, T., Schröder, A., Lu, M., *et al.*, *FASEB J.* 2001, *15*, 403 – 415.

[7] Weber, T., Lu, M., Andera, L., Lahm, H., *et al.*, *Clin. Cancer Res.* 2002, *8*, 863 – 869.

[8] Birringer, M., EyTina, J. H., Salvatore, B. A., Neuzil, J., *Br. J. Cancer* 2003, *88*, 1948 – 1955.

[9] Neuzil, J., Zhao, M., Ostermann, G., Sticha, M., *et al.*, *Biochem. J.* 2002, *362*, 709 – 715.

[10] Estronca, L. M., Moreno, M. J., Abreu, M. S., Melo, E., Vaz, W. L., *Biochem. Biophys. Res. Commun.* 2002, *296*, 596 – 603.

[11] Arya, P., Alibhai, N., Qin, H., Burton, G. W., *et al.*, *Bioorg. Med. Chem. Lett.* 1998, *8*, 2433 – 2438.

[12] Chinery, R., Brockman, J. A., Peeler, M. O., Shyr, Y., *et al.*, *Nat. Med.* 1997, *3*, 1233 – 1241.

[13] Yu, W., Simmons-Menchaca, M., Gapor, A., Sanders, B. G., Kline, K., *Nutr. Cancer* 1999, *33*, 26 – 32.

[14] McIntyre, B. S., Briski, K. P., Tirmenstein, M. A., Fariss, M. W., *et al.*, *Lipids* 2000, *35*, 171 – 180.

[15] Wada, S., Satomi, Y., Murakoshi, M., Noguchi, N., *et al.*, *Cancer Lett.* 2005, *229*, 181 – 191.

[16] Birringer, M., Drogan, D., Brigelius-Flohe, R., *Free Radic. Biol. Med.* 2001, *31*, 226 – 232.

[17] Tomic-Vatic, A., EyTina, J. H., Chapmann, J. M., Mahdavian, E., *et al.*, *Int. J. Cancer* 2005, *117*, 118 – 193.

[18] Wang, X. F., Witting, P. K., Salvatore, B. A., Neuzil, J., *Biochem. Biophys. Res. Commun.* 2005, *326*, 282 – 289.

[19] Swettenham, E., Witting, P. K., Salvatore, B. A., Neuzil, J., *J. Neurochem.* 2005, *94*, 1448 – 1456.

[20] Fariss, M. W., Fortuna, M. B., Everett, C. K., Smith, J. D., *et al.*, *Cancer Res.* 1994, *54*, 3346 – 3351.

[21] Wu, Y., Zu, K., Ni, J., Yeh, S., *et al.*, *Anticancer Res.* 2004, *24*, 3795 – 3802.

[22] Neuzil, J., *Biochem. Biophys. Res. Commun.* 2002, *24*, 1309 – 1313.

[23] Neuzil, J., Massa, H., *Biochem. Biophys. Res. Commun.* 2005, *327*, 1024 – 1027.

[24] Green, D. R., Kroemer, G., *Science* 2004, *305*, 626 – 629.

[25] Weber, T., Dalen, H., Andera, L., Nègre-Salvayre, A., *et al.*, *Biochemistry* 2003, *42*, 4277 – 4291.

[26] Dey, R., Moraes, C. T., *J. Biol. Chem.* 2000, *275*, 7087 – 7094.

[27] Higuchi, M., Aggarwal, B. B., Yeh, E. T., *J. Clin. Invest.* 1997, *99*, 1751 – 1758.

[28] Cory, S., Huang, D. C., Adams, J. M., *Oncogene* 2003, *22*, 8590 – 8607.

[29] Yamamoto, S., Tamai, H., Ishisaka, R., Kanno, T., *et al.*, *Free Radic. Res.* 2000, *33*, 407 – 418.

[30] Yu, W., Sanders, B. G., Kline, K., *Cancer Res.* 2003, *63*, 2483 – 2491.

[31] Ruvolo, P. P., Deng, X., Carr, B. K., May, W. S., *J. Biol. Chem.* 1998, *273*, 25436 – 25442.

[32] Du, C., Fang, M., Li, Y., Li, L., Wang, X., *Cell* 2000, *102*, 33 – 42.

[33] Verhagen, A. M., Vaux, D. L., *Apoptosis* 2002, *7*, 163 – 166.

[34] Erl, W., Weber, C., Wardemann, C., Weber, P. C., *Am. J. Physiol.* 1997, *273*, H634 – H640.

[35] Neuzil, J., Schröder, A., von Hundelshausen, P., Zernecke, A., *et al.*, *Biochemistry* 2001, *40*, 4686 – 4692.

[36] Dalen, H., Neuzil, J., *Br. J. Cancer* 2003, *88*, 153 – 158.

[37] Susin, S. A., Lorenzo, H. K., Zamzami, N., Marzo, I., *et al.*, *Nature* 1999, *397*, 441 – 446.

[38] Cande, C., Cecconi, F., Dessen, P., Kroemer, G., *J. Cell Sci.* 2002, *115*, 4727 – 4734.

[39] Ottino, P., Duncan, J. R., *Free Radic. Biol. Med. 22*, 1145 – 1151.

[40] Kogure, K., Morita, M., Nakashima, S., Hama, S., *et al.*, *Biochim. Biophys. Acta* 2001, *1528*, 25 – 30.

[41] Kogure, K., Hama, S., Manabe, S., Tokumura, A., Fukuzawa, K., *Cancer Lett.* 2002, *186*, 151 – 156.

[42] Kang, Y. H., Lee, E., Choi, M. K., Ku, J. L., *et al.*, *Int. J. Cancer* 2004, *112*, 385 – 392.

[43] Stapelberg, M., Gellert, N., Swettenham, E., Tomasetti, M., *et al.*, *J. Biol. Chem.* 2005, *280*, 25369 – 25376.

[44] Kelso, J. F., Porteous, C. M., Coulter, C. V., Hughes, G., *et al.*, *J. Biol. Chem.* 2001, *276*, 4588 – 4596.

[45] Alleva, R., Tomasetti, M., Andera, L., Gellert, N., *et al.*, *FEBS Lett.* 2001, *503*, 46 – 50.

[46] Ogretmen, B., Hannun, Y. A., *Nat. Rev. Cancer* 2004, *4*, 604 – 616.

[47] Dimanche-Boitrel, M. T., Meurette, O., Rebillard, A., Lacour, S., *Drug Resist. Updat.* 2005, *8*, 5 – 14.

[48] Ruvolo, P. P., Deng, X., Ito, T., Carr, B. K., May, W. S., *J. Biol. Chem.* 1999, *274*, 20296 – 20300.

[49] Brunk, U. T., Neuzil, J., Eaton, J., *Redox Report* 2001, *6*, 91 – 97.

[50] Neuzil, J., Svensson, I., Weber, T., Weber, C., Brunk, U. T., *FEBS Lett.* 1999, *445*, 295 – 300.

[51] Terman, A., Neuzil, J., Kågedal, K., Öllinger, K., Brunk, U. T., *Exp. Cell Res.* 2002, *274*, 9 – 15.

[52] Takahashi, K., Loo, G., *Biochem. Pharmacol.* 2004, *67*, 315 – 324.

[53] Mo, H., Elson, C. E., *J. Nutr.* 1999, *129*, 804 – 813.

[54] Shah, S. J., Sylvester, P. W., *Biochem. Cell Biol.* 2005, *83*, 86 – 95.

[55] Roskoski, R., *Biochem. Biophys. Res. Commun.* 2002, *319*, 1 – 11.

[56] Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., *et al.*, *Science* 1989, *244*, 707 – 712.

[57] Zhou, B. P., Hung, M. C., *Semin. Oncol.* 2003, *30*, 38 – 48.

[58] Vivanco, I., Sawyers, C. L., *Nat. Rev. Cancer* 2002, *2*, 489 – 501.

[59] Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., *et al.*, *Science* 1997, *275*, 661 – 665.

[60] Datta, S. R., Dudek, H., Tao, X., Masters, S., *et al.*, *Cell* 1997, *9*, 231 – 241.

[61] Cardone, M. H., Roy, N., Stennicke, H. R., Salvesen, G. S., *et al.*, *Science* 1998, *282*, 1318 – 1321.

[62] Kane, L. P., Shapiro, V. S., Stokoe, D., Weiss, A., *et al.*, *Curr. Biol.* 1999, *9*, 601 – 604.

[63] LaCasse, E. C., Baird, S., Korneluk, R. G., MacKenzie, A. E., *et al.*, *Oncogene* 1998, *17*, 3247 – 3259.

[64] Akazawa, A., Nishikawa, K., Suzuki, K., Asano, R., *et al.*, *Jpn. J. Pharmacol.* 2002, *89*, 417 – 421.

[65] Yu, W., Liao, Q. Y., Hantash, F. M., Sanders, B. G., Kline, K., *et al.*, *Cancer Res.* 2001, *61*, 6569 – 6576.

[66] Wu, K., Zhao, Y., Li, G. C., Yu, W. P., *World J. Gastroenterol.* 2004, *10*, 1110 – 1114.

[67] Zu, K., Hawthorn, L., *Mol. Cancer Ther.* 2005, *4*, 43 – 50.

[68] Levkau, B., Scatena, M., Giachelli, C. M., Ross, R., Raines, E. W., *Nat. Cell Biol.* 1999, *1*, 227 – 233.

[69] Harvey, K. J., Lukovic, D., Ucker, D. S., *J. Cell Biol.* 2000, *148*, 59 – 72.

[70] Ni, J., Chen, M., Zhang, Y., Li, R., *et al.*, *Biochem. Biophys. Res. Commun.* 2003, *300*, 357 – 363.

[71] Alleva, R., Benassi, M. S., Tomasetti, M., Gellert, N., *et al.*, *Cancer Lett.* 2006, *232*, 226 – 253.

[72] Turley, J. M., Ruscetti, F. W., Kim, S. J., Fu, T., *et al.*, *Cancer Res.* 1997, *57*, 2668 – 2675.

[73] Tomasetti, M., Rippo, M. R., Alleva, R., Moretti, S., *et al.*, *Br. J. Cancer* 2004, *90*, 1644 – 1653.

[74] Stapelberg, M., Tomasetti, M., Gellert, N., Alleva, R., *et al.*, *Biochem. Biophys. Res. Commun.* 2004, *318*, 636 – 641.

[75] Woodson, K., Tangrea, J. A., Barrett, M. J., Virtamo, J., *et al.*, *J. Natl. Cancer Inst.* 1999, *91*, 1738 – 1743.

[76] Rautalahti, M. T., Virtamo, J. R., Taylor, P. R., Heinonen, O. P., *et al.*, *Cancer* 1999, *86*, 37 – 42.

[77] Malila, N., Taylor, P. R., Virtanen, M. J., Korhonen, P., *et al.*, *Cancer Causes Control* 2002, *13*, 617 – 623.

[78] Lippman, S. M., Goodman, P. J., Klein, E. A., Parnes, H. L., *et al.*, *J. Natl. Cancer Inst.* 2005, *97*, 94 – 102.

[79] Zu, K., *Cancer Res.* 2003, *63*, 6988 – 6995.

[80] Vraka, P. S., Drouza, C., Rikkou, M. P., Odysseos, A. D., Keramidas, A. D. *Bioorg. Med. Chem.* 2006, *14*, 2684 – 2696.

[81] Neuzil, J., Weber, T., Gellert, N., Weber, C., *Br. J. Cancer* 2001, *84*, 87 – 89.

[82] Wu, K., Zhao, Y., Liu, B. H., Li, Y., *et al.*, *World J. Gastroenterol.* 2002, *8*, 26 – 30.

[83] Hrzenjak, A., Reicher, H., Wintersperger, A., Steinecker-Frohnwieser, B., *et al.*, *Cell. Mol. Life Sci.* 2004, *61*, 1520 – 1531.

[84] Anderson, K., Simmons-Menchaca, M., Lawson, K. A., Atkinson, J., *et al.*, *Cancer Res.* 2004, *64*, 4263 – 4269.

[85] Bang, O. S., Park, J. H., Kang, S. S., *Biochem. Biophys. Res. Commun.* 2001, *288*, 789 – 797.

[86] Israel, K., Yu, W., Sanders, B. G., Kline, K., *Nutr. Cancer* 2000, *36*, 90 – 100.

[87] Wu, K., Shan, Y. J., Zhao, Y., Yu, J. W., Liu, B. H., *World J. Gastroenterol.* 2001, *7*, 60 – 64.

[88] Helson, L., Verma, M., Helson, C., in: Prasad, K. N. (Ed.), *Modulation and Mediation of Cancer by Vitamins*, Karger, Basel 1983, pp. 258 – 265.

[89] Malafa, M. P., Neitzel, L. T., *J. Surg. Res.* 2000, *93*, 163 – 170.

[90] Malafa, M. P., Fokum, F. D., Mowlavi, A., Abusief, M., King, M., *Surgery* 2002a, *131*, 85 – 91.

[91] Barnett, K. T., Fokum, F. D., Malafa, M. P., *J. Surg. Res.* 2002, *106*, 292 – 298.

[92] Tomasetti, M., Gellert, N., Procopio, A., Neuzil, J., *Int. J. Cancer* 2004, *109*, 641 – 642.

[93] Malafa, M. P., Fokum, F. D., Smith, L., Louis, A., *Ann. Surg. Oncol.* 2002, *85*, 1023 – 1032.

[94] Borel, P., Pasquier, B., Armand, M., Tyssandier, V., *et al.*, *Am. J. Physiol. Gastrointest. Liver Physiol.* 2001, *280*, G95 – G103.

[95] Lawson, K. A., Anderson, K., Simmons-Menchaca, M., Atkinson, J., *et al.*, *Exp. Biol. Med.* 2004, *229*, 954 – 963.

[96] Kaempf-Rotzoll, D. E., Traber, M. G., Arai, H., *Curr. Opin. Lipidol.* 2003, *14*, 249 – 254.

[97] Traber, M. G., *Curr. Opin. Gastroenterol.* 2005, *21*, 223 – 227.

[98] Kessler, M., Ubeaud, G., Walter, T., Sturm, F., Jung, L., *J. Dermatol. Treat.* 2002, *13*, 133 – 141.

[99] Miller, E. R., Pastor-Barriuso, R., Dala, D., Riemersma, R. A., *et al.*, *Ann. Intern. Med.* 2005, *142*, 37 – 46.

[100] Ford, E. S., Ajani, U. A., Mokhad, A. H., *Ann. Intern. Med.* 2005, *143*, 116 – 120.

[101] Guallar, E., Hanley, D. F., Miller, E. R., *Ann. Intern. Med.* 2005, *143*, 143 – 145.

[102] Zhang, S., Lawson, K. A., Simmons-Menchaca, M., Atkinson, J., *et al.*, *Breast Cancer Res. Treat.* 2004, *87*, 111 – 121.

[103] Ramanathapuram, L. V., Hahn, T., Graner, M. W., Katsanis, M., Akporiaye, E. T., *Cancer Immunol. Immunother.* 2006, *55*, 166 – 177.

