·Review·

Anticancer mechanisms of vitamin E succinate

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[Abstract] Vitamin E succinate (RRR-α-tocopheryl succinate, VES) is an ester derivative of vitamin E. Roles of vitamin E (α-tocopherol) family in cancer prevention and therapy have been investigated since 1960s'. Experimental evidences indicated that VES is one of the most effective anticancer compounds of the vitamin E family. VES can effectively inhibit many kinds of tumors without toxic effects on normal cells and tissues. This article reviewed the anticancer mechanisms of VES in the following four aspects: 1) the molecular structure, chemical property and carrier of VES; 2) the mechanisms of VES in inhibiting cancer cell proliferation; 3) the mechanisms of VES-induced apoptosis of cancer cells; 4) the mechanisms of VES in preventing tumor metastasis. Investigation on the anticancer mechanisms of VES would help find new targets and develop new effective and safe drugs for cancer prevention and treatment.

Key words: Vitamin E succinate, α-tocopheryl succinate, cancer prevention, cell apoptosis, anticancer mechanism

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Despite the advances in medicine, cancer remains one of the leading causes of death in humans. The World Health Organization reported that about 7.6 million people around the world died from cancer each year. The traditional anti-cancer concept is "searching and eliminating," i.e., diagnosis and treatment are started after the onset of cancer. However, this strategy cannot effectively control the incidence and mortality of cancer. Thus, modern anti-cancer concept has evolved into "targeting and controlling". One of the strategies is to adjust and revere cancer lesions by chemical prevention, thereby to reduce incidence and mortality of cancer.

Vitamin E succinate (RRR-α-tocopheryl succinate, VES) is an ester derivative of vitamin E (VE) that has received much attention from many researchers for its potential in chemoprevention in recent years. Many studies found that VES had broad anti-tumor effects, without toxicity to normal human cells and tissues.[1-4] VES can selectively induce tumor cell apoptosis and inhibit tumor cell proliferation at the cellular level, thereby inhibit growth, invasion and metastasis of tumor at the tissue level. This highly effective and low toxic anti-tumor property of VES makes it an ideal anti-cancer drug candidate, so many studies focusing on anti-tumor effects and mechanisms of VES have been performed in recent years. Some studies investigated inhibition on cell cycle and DNA synthesis by VES,[5-10] while the majority of studies focused on signaling pathways of VES-induced apoptosis and its inhibition on tumor metastasis.[10-27] This review summarized literature on anti-tumor mechanisms of VES before March 2009 and discussed anti-tumor effects

of VES from the following four aspects: 1) the molecular structure, chemical property and carrier of VES; 2) the mechanisms of VES in inhibiting tumor cell proliferation; 3) the mechanisms of VES-induced tumor cell apoptosis; and 4) the mechanisms of VES in preventing tumor metastasis. In-depth study of anticancer mechanisms of VES should help not only to evaluate accurately the clinical value of VES as a tumor prevention and treatment drug, but also to further understand the nature of tumor in order to provide reference for the development of chemoprevention and treatment drugs for tumor.

Molecular structure, chemical properties and carriers of VES

The anti-tumor properties of VES are related to its specific molecular structure, chemical properties and carrier.[28-30] Lipoprotein and tocopherol-binding protein-1 may respectively act as the systematic and intracellular carriers for VES.[31,32]

Molecular structure and chemical properties. The molecular structure of VES can be divided into three domains: hydrophobic domain I, signaling domain II and functional domain III,[28] and the antitumor effects of VES are closely related to each domain in its molecular structure. The hydrophobic domain contains a phytyl chain, mainly involved in binding capacity of VES to cell membrane and its lipoprotein carrier.[28] The signaling domain contains benzodihydropyran, which is related to regulation of protein kinase C (PKC) activity by VES.[29] The specific functional domain of VES is a charged succinate group, an essential structure for induction of cell apoptosis,[29] since VE lacking this structure does not have this function. β and γ-isomers of VES and cholesteryl succinate cannot regulate activity of PKC, but can induce cell apoptosis,[30] indicating the significance of the succinate structure in VES for its pro-apoptotic function on tumor cells. Thus, the molecular structure of VES is the basis for its anti-tumor properties.

Some studies showed that VES can selectively inhibit tumor cell proliferation and induce tumor cell apoptosis, but has no toxicity to normal tissues and cells.[1-4] This specific anti-tumor effect of VES may be related to its chemical properties. VES exists in solution in two species: deprotonated charged form and protonated uncharged form and the latter can enter cell through free diffusion. Therefore, bioavailability of VES inside cell depends on the ratio of these two species, which is largely determined by pH of the environment. VES is a weakly acidic reagent, with a pKα of 5.64. The Henderson-Hasselbalch equation states: $pH = pKa + \log([COO^-]/[COOH])$. In a neutral environment, about 99% of VES are charged, while at pH6.2, uncharged form accounts for up to 25% of VES. The interstitium of tumor cells is acidic, which allows free diffusion of VES into tumor cells in large quantity, while normal cells have neutral interstitium and cannot easily uptake charged VES.[33] Study results by Neuzil et al.[30] also provided strong support for this theory, which showed that VES could more effectively induce cell apoptosis in leukemia Jurkat cell and breast cancer MCF-7 cell at lower pH, and its pro-apoptotic activity was pH-dependent since this ability decreased along with lowered pH.

Transport carrier. VES is a hydrophobic compound, and thus, it requires transport carrier to move intracellularly to target such as mitochondria. Lipoprotein and tocopherol-associated protein-1 (TAP-1) are probably the VES system and the intracellular carrier, respectively.[31,32]

Research showed that the level of VES in tissues primarily depended on the amount of lipoprotein. The binding efficiency of lipoprotein with VES was determined by the size of lipoprotein microgranule and the amount of triglyceride in lipoprotein. The absorption rate of lipoprotein with VES depended on the expression level of lipoprotein receptors. The VES, which was binded to very low density lipoprotein (VLDL) and low density lipoprotein (LDL), when compared with free VES, was more effective in inhibiting the growth of tumor cells MCF-7 of breast cancer.[31]

Tocopherol-associated protein-1 (TAP-1) is a class of proteins with high affinity for vitamin E and it exists in many tissues and cells. It is recognized as the transport carrier for the family of vitamin E in the body. The research results for malignant mesothelioma demonstrated that the cells of malignant mesothelioma after transfection of plasmid with TAP-1 would express TAP-1 excessively. These cells underwent apoptosis when VES of lower dosage (10-25M) was applied. On the other hand, under the same dosage of VES, cells of malignant mesothelioma without transfection of plasmid with TAP-1 did not show apoptosis. In these transfected cells of malignant mesothelioma with high expression level of TAP1, VES was accumulated to a much

higher level. This enhanced effect of promoting apoptosis due to TAP1, as exhibited by VES, was related to instability of mitochondria and activation of caspase. These results suggested that VES had higher affinity to bind to TAP-1, where TAP-1 served as an intracellular carrier for VES to transport it into mitochondria, in order to strengthen VES to induce apoptosis in tumor cells.[32]

The inhibitory mechanism on tumor cell proliferation by vitamin E succinate

Tumor is the byproduct of unlimited cell proliferation after cell cycle became uncontrolled. From the molecular perspective, it is the genetic mutation, which causes the hyperactive activation of promotion factors for cell cycle (or known as cancer protein) OR/AND the deactivation of inhibitory factors (known as inhibitory cancer protein). These cause regulation on cell cycle to dysfunction. The mechanisms by VES to inhibit tumor cell proliferation involve: inhibition on DNA synthesis, delay of cell cycle, and control of regulatory proteins for cell cycle.[8-10] In addition, VES can, through regulation on tumor -related factors, such as Ras gene, transcription factor NF-B, and AP-1, inhibit tumor cell proliferation.[2,34-35]

Cell cycle. According to the report by Gu et al.,[10] five different tumor cells of head and neck (JHU-011, 013, 019, 022, 029) were stopped at S-phase and G1-phase after they were treated with VES in culture media for 24 hours. In the report by Yu et. al., 5in tumor cells MDA-MB-435 of breast cancer, VES inhibited DNA synthesis and blocked tumor cells at G0/G1 phase of the cell cycle. Also, this effect was dosage and time dependent. $P21^{Waf1/Cip}$ is an important regulator for cell cycle and its function is to block cell cycle. In MDA-MB-435 cells, which were treated with VES, the expression levels of mRNA and protein of $P21^{Waf1/Cip}$ were up-regulated. Also, the antisense oligomers of P21 could stop the growth inhibition by VES. Furthermore, some researches discovered that the antisense oligomers of $P21^{Waf1/Cip}$ could stop the cell differentiation of MDA-MB-435, suggesting that VES inhibited DNA synthesis in MDA-MB-435 cells of breast cancer by activating $P21^{Waf1/Cip}$, while this inhibition was based on VES -induced apoptotic TGF- signal pathway.[8]

Ni et al.[9] discovered that VES inhibited the growth and proliferation of LNCaP tumor cells of prostate cancer and blocked it at G_1 phase of cell cycle. The underlying mechanism might involve the reduction in expression levels of regulatory proteins for cell cycle, such as Cyclin D1, Cyclin D3, Cyclin E, CDK2, and CDK4. In addition, VES could lower the activities of CDK4 kinase, the phosphorylation of Rb, and the expression of mRNA of Cyclin E. Therefore, it could be seen that the regulation on multiple regulatory factors for the cell cycle by VES led to blockage on LNCaP cells at G_1/S phase.

Ras gene. The oncogene Ras, when overly expressed, can lead to continuous proliferation of tumor cells. The inhibitory effect of VES on tumor cell proliferation relies on an important pathway through regulating Ras. Donapaty et al.[34] found that VES could inhibit cell proliferation of NIH3T3 with stable transfected oncogenes of K -Ras and H -Ras to induce apoptosis. On the other hand, it had no effect on transfected NIH3T3 cells with empty carrier. In NIH3T3 cells with expressing Ras, the expressions of transcriptional targets of Ras, such as c-Myc, cyclin D1, and E2F1, were down-regulated by VES. In HCT116 tumor cells of human colon cancer and MDA-MB-231 tumor cells of human breast cancer, which had expression of K-Ras, VES exhibited the same down-regulating effect.

Transcriptional factors NF -B and AP -1. VES regulates transcriptional factors, NF-B and AP-1 to inhibit tumor cell proliferation. Crispen et al.[2] discovered in non -testosterone dependent prostate cancer cells PC-3, DU-145, and CA-HPV-10, VES could inhibit the activities of NF-B and enhance the activities of AP-1. It down-regulates the expressions of interleukin-6 (IL-6), interleukin-8 (IL-8), and VEGF. In addition, it also lowers the expressions of anti-apoptosis proteins, XIAP and Bcl-2. Dalen et al. 35 found that in Jurkat lymphoma, VES inhibited the activities of NF -B to sensitize Jurkat cells to the process of TRAIL-dependent apoptosis.

The mechanism of inducing apoptosis in tumor cells by vitamin E. succinate

Cell proliferation and cell apoptosis is a pair of basic systems to maintain basic normal physiological conditions in a body. The incidence of tumor is primarily the result of dysfunction in these two systems. Research showed that VES induced

apoptosis in tumor cells through regulation of multiple signal pathways,[10–27,36–37] the primary two of which were the extrinsic receptor–related pathway and the intrinsic mitochondrial pathway. The extrinsic pathway will induce apoptosis by binding of extracellular signal molecule with transmembrane receptor, while the intrinsic pathway involves the activation of stimulus for death inside a cell, which manifests as mitochondrial dysfunction. The extrinsic and the intrinsic pathway respectively activate caspase–8 and caspase–9 for initiation of apoptotic process. Extrinsic and intrinsic pathways are not independent of each other, but there is a certain degree of interaction between the two. MAPK pathway plays a role of connector for extrinsic and intrinsic pathway during the apoptotic process by VES, where the activation of JNK is regulated by extrinsic pathway and it influences the phosphorylation of Bcl–2 in the intrinsic pathway, thus affecting the migration of Bax from cytoplasm to mitochondria. It certainly plays an important role in the intrinsic pathway. PKC pathway, by regulating phosphorylation of Bcl–2, contributes to the apoptotic process by VES.

Extrinsic pathway. Fas pathway. The death receptor, Fas, is a member of the superfamily of neural growth factor and tumor necrosis factor receptor (NFG/TNF), which is primarily expressed on the surface of activated T–cell, where its agonistic antibody binds to induce apoptosis in tumor cells. When Fas is stimulated by agonistic antibody in dissolving form on the external side of cell membrane, it leads to apoptosis. Some reports claimed that VES–regulated apoptosis involved the Fas death receptor pathway.[19,36–37] In MDA–MB–231 and SKBR–3 cells, which were treated with VES, the expressions of Fas receptor and Fas–L protein were increased, while VES further promoted expression of Fas on cell surface. In addition, neutral antibody and antisense nucleic acid of Fas could prevent apoptosis induced by VES. The antisense nucleic acid of Fas–L could also prevent VES–regulated increase in protein expression of Fas–L. Therefore, VES could induce apoptosis in MDA–MB–231 and SKBR–3 cells of breast cancer with negative estrogen receptor by activating Fas/Fas–L pathway.[36] Research showed that VES transformed the tumor cells of breast cancer from being Fas–tolerant to Fas–sensitive and this kind of regulation of VES was related to the relocation of Mr43000 Fas from cytoplasm to cell surface membrane.[37] In SGC–7901 gastric tumor cells, which were treated with VES, the expressions of Fas, Fas–associating protein with death domain (FADD) and caspase–8 were elevated. Also, the antisense nucleic acid of transfected Fas could significantly inhibit the expression of FADD, while the activities of caspase–8 were dramatically reduced, too. Therefore, the VES–induced apoptosis in gastric tumor cells SGC–7901 involved the Fas signal pathway, in which it included interaction among Fas, FADD, and caspase–8.[19]

TGF– pathway. Transforming growth factor–(TGF–) is a cytokine with multiple biological functions, which rely on the receptors on cell membrane, including inhibition on cell proliferation and induction of epithelial apoptosis. There are three known types of TGF– receptor, in which type I and II participate in signal transduction of TGF–, while type III regulates the binding of TGF– to signal transduction receptor, even though it is not involved in the signal transduction process. Research found that TGF– pathway was non–functional in MDA–MB–435 tumor cells of human breast cancer, but it was restored after being treated with VES. VES could restore TGF– pathway by transforming potential and inactive TGF– into active ligand and up–regulating the expression of TGF– R–II on cell surface membrane.[20] Wu et al.[38] also discovered that after gastric tumor cells SGC–7901 were treated with VES, the expressions of TGF–, JNK, and c–Jun increased. Therefore, TGF– pathway played a role in VES–induced apoptosis for tumor cells. Recent research found that the treatment with VES would inhibit growth of prostate cancer cells PC3 and induce their apoptosis. Meanwhile, the protein and mRNA of a member of TGF– superfamily, NAG–1, were up–regulated in a dosage–dependent manner. Also, this was a P38–kinase dependent regulation. As a result, NAG–1 could be a target point for chemical prevention and treatment in prostate cancer.[39]

Intrinsic pathway. Current research proves that VES primarily induces apoptosis in tumor cells through intrinsic pathway.[10,12,22–27] Intrinsic pathway is triggered by death–related stimuli within a body, including reactive oxygen species, cytochrome C, Bcl–2 family protein, sphingomyelinase (SMase), and spongiamine. The apoptosis of intrinsic origin will manifest as dysfunctional mitochondria and is, to a certain degree, regulated by the extrinsic pathway. In the early stage of VES application, VES induces tumor cells to produce reactive oxygen species

(ROS) and spongiamine. These activities cause changes in mitochondrial morphology and functions, which further leads to release of cytochrome C into cytoplasm by mitochondria. Cytochrome C will bind with Apaf-1 to form a complex, which is further joined by pro-caspase-9, before the entire complex is activated when Apaf-1 recruits these domains. The pro-caspase-9 breaks free and activates downstream effectors such as caspase-3, caspase-6 and caspase-7, in order to amplify a cascade reaction of caspases that initiates the process of apoptosis.

Reactive oxygen species. Reactive oxygen species are also known as free radical of oxygen and they are closely related to the process of apoptosis. It is primarily generated in endoplasmic reticulum, external membrane of mitochondria, and nuclear membrane. The generation of reactive oxygen species has great significance in the apoptosis, involving mitochondria. It participates in the initial stage of drug induction and receptor-mediated apoptosis. Research discovered that VES could cause generation of reactive oxygen species in many types of tumor cells.[12,16,40-42] It was possibly the early reaction to VE-associated compounds from tumor cells. In Jurkat T. lymphoma, generation of reactive oxygen species could be observed in one hour after the cells were treated with VES.[24] The reactive oxygen species in tumor cells from VES treatment mostly existed in the form of superoxide compound, where application of superoxide dismutase (SOD) could remove these free radicals and inhibit cell death [24, 40]. The location for generation of superoxide compounds and the binding site for reactive oxygen species are suspected to be mitochondria. Some experiments showed the coenzyme Q, which used mitochondria as the target site, inhibited the accumulation of reactive oxygen species and VES-induced apoptosis.[12,24] There were reports claiming that in tumor cells with reduced oxidation ability, the phenomenon of VEs-induced apoptosis was more profound.[39] One of the major factors for intracellular generation of reactive oxygen species was complex II from mitochondrial respiratory chain,[25] even though its underlying mechanism still required further investigation.

Sphingomyelinase and spongiamine. Spongiamine is an important metabolic product of sphingolipids and it plays an important role in the process of apoptosis for many types of tumor cells.[40] VES can induce apoptosis after it increases the activities of sphingomyelinase (SMase) to activate spongiamine. Gu et al.[10] found that when VES was used to treat tumor cells of head and neck (JHU22), the activities of SMase on cell membrane were the first to be boosted. SMase could hydrolyze sphinogomyelin to increase the concentration of spongiamine in cells, and thus, the spongiamine-related apoptotic pathway was initiated. In addition, spongiamine-mediated apoptotic signal could affect the expressions of caspase-3 and Bcl-2 family proteins. SMase was the possible, direct target site of VES. When VES was used to treat Jurkat cells in leukemia, it caused SMase to be activated in 15 to 30 minutes and this activation was not inhibited by the inhibitory factors of caspase.[16] The activation of SMase could be caused by the binding of lipophilic VES, where there was a change in membrane fluidity. In cells treated with VES, VES induced synthesis of spongiamine to activate PP2A. This result was in accordance to the finding where long-chain spongiamine was found to be the activating factor for PP2A. There was report that VES, in Jurkat cells, activated sphingomyelinase to alter mitochondrial structure.[39] The mitochondria-dependent activities, which were induced by apoptotic process, were probably caused by direct influence on mitochondria from VES or/and generation of spongiamine. These processes exerted some influences on the instability of mitochondrial membrane.[18, 35]

Apoptotic factor such as cytochrome C. In the apoptotic process induced by VES-associated compound, unstable downstream activities in mitochondria include migration of various apoptotic regulatory factors such as cytochrome C and Smac/Diablo.[43] The relocation of cytochrome C from mitochondria to cytoplasm triggers a cascade reaction of caspase, pushing cell to enter apoptotic process.[16] Smac/Diablo is an important stimulus for caspase-dependent apoptotic signal, as it acts as an antagonist against caspase inhibitor in the family of inhibitory apoptotic proteins (IAPs). The expression of IAPs is controlled by NF-B, while the activities of NF-B are inhibited by VES.[34] Therefore, relocation of Smac/Diablo in cell can further promote an inhibition on the survival signal pathway in a VES-induced apoptotic cell.[24]

Bcl-2 family proteins. Bcl-2 family members are key regulatory factors in the intrinsic pathway.[44-45] They directly control the permeability of mitochondrial

membrane to regulate the release of cytochrome C. According to the homogeneous domains and functions in Bcl-2, the Bcl-2 family proteins can be divided into three categories: anti-apoptotic members with four types of BH functional domains such as Bcl-2 and Bcl-xL; pro-apoptotic members with BH1/BH2/BH3 domains such as Bax and Bak; members with only BH3 functional domain such as Bad and Bim. When apoptotic signal is received, Bax and Bak form a heterogeneous tetrapolymer channel for release of cytochrome C. On the contrary, Bcl-2 and Bcl-xL prevent the formation of this heterogeneous tetrapolymer channel by Bax and Bak. 22

Bcl-2 family proteins play an important role in VES-induced apoptosis.[22,26-27] VES can inhibit the functions of Bcl-2 and Bcl-xL to induce apoptosis in prostate cancer cells. The in vitro experiment demonstrated that VES could destroy the bonds-BH3 peptide of Bak to Bcl-2 and Bcl-xL. This resulted in caspase-dependent cell apoptosis.[26] Yu et al.[27] believed that the process of VES-induced apoptosis included activation of JNK, structural change in Bax, migration of Bax from cytoplasm to mitochondria, opening of mitochondrial permeability transition pore (MPT), release of cytochrome C to cytoplasm from mitochondria, activation of caspase-3/caspase-9, and lysis of PARP. In MDA-MB-435 tumor cells of breast cancer, which were treated with VES, there was change in mitochondrial permeability. Also, in comparison to cells treated with VE, there was a decrease in level of Bax in cytoplasm of tumor cells with VES and an elevation in cytochrome C. However, in the total extraction from cell, the levels of cytochrome C and Bax were unchanged. These phenomena suggested that in breast cancer cells, VES caused migration of Bax from cytoplasm to mitochondria and this was probably the reason for release of cytochrome C from mitochondria.

Other pathways. Cellular apoptosis, which is induced by VES, also involves MAPK and PKC pathway. MAPK serves as a connection between the extrinsic and the intrinsic pathway during VES-induced apoptosis. The member of MAPK family, JNK, by regulating phosphorylation of Bcl-2 and migration of Bax from cytoplasm to mitochondria, connects the extrinsic and the intrinsic pathway. The PKC pathway plays a role in the VES-induced apoptosis by direct and indirect regulation on phosphorylation of Bcl-2.

MAPK pathway. Mitogen-activated protein kinase (MAPK) pathway is one of the important pathways in the network of signal transduction for eukaryotic organisms. It also plays a role in the VES-induced apoptosis. MAPK chain is composed of three types of protein kinase: MAP3K-MAP2K-MAPK, and through orderly phosphorylation, the upstream signal is relayed to downstream effector. MAPK signal pathway includes: MAP kinase (MAPK), MAPK kinase (MEK, MKK, or MAPK kinase), and MEK kinase (MEKK, MKKK, or MAPK kinase). The c-Jun, a kinase at the N-terminal (JNK), is a member of MAPK family. JNK is the most important kinase for phosphorylation of c-Jun, in which it can enhance the activities of transcriptional factor AP-1. Research showed that VES could promote the activities of ERK1/2 and JNK, even though it proved to be non-effective to P38 kinase.[21] The research by Zu et al.[22] discovered that in prostate cancer cells, the three upstream components: apoptotic signal regulatory kinase-1 (Ask-1), growth arrest and DNA damage gene (GADD45), and activated protein kinase/ERJ kinase-1 by stress, in the cascade reaction of JNK could all be activated by VES, while VES could also improve the expression of phosphorylated JNK proteins.

In the VES-induced apoptosis, JNK receives regulation from extrinsic pathway and it is able to control intrinsic pathway by regulating phosphorylation of Bcl-2 and migration of Bax from cytoplasm to mitochondria. JNK can reduce the activities of apoptosis by phosphorylation of Bcl-2, as well as play a role in the migration of Bax from cytoplasm to mitochondria. The activation of JNK causes phosphorylation of Bim, which further alters structure of Bax and leads to its migration into mitochondria. The activation of JNK can directly alter structure of Bax and allows migration of Bax into mitochondria from cytoplasm, which leads to a change in mitochondrial permeability.[22]

PKC pathway. PKC pathway is an important component in the process of apoptosis. Currently, there are different perspectives on the effect of PKC in the VES-induced apoptosis. Neuzil et al.[18] found that VES activated PP2A, which further dephosphorylate PKC to loss of activeness. The deactivation of PKC caused dephosphorylation of Bcl-2. PP2A could also directly dephosphorylate Bcl-2. In addition, the electrically charged part succinate of VES caused instability in organelles

such as lysosome and mitochondria. The instability in these organelles, activation of PP2A and deactivation of PKC worked together to induce release of cytochrome C from mitochondria. Cytochrome C formed apoptotic body with apaf-1 and caspase-9. The mature caspase-9 was released and it activated other caspases, which were in charge of execution of apoptotic process, such as caspase-3. These finally led to apoptosis. Bang et al.[23] found that in HL-60 cells of human leukemia, the activation of PKC was rather necessary for VES to induce apoptosis. The possible reason for the effect of PKC on VES-induced apoptosis might have to do with the existence of specific isoenzymes of heterogeneous origin for PKC, which in turn causes PKC to have different or sometimes even opposite effects.

The mechanism for Vitamin E. succinate to inhibit metastasis

Metastasis refers to the migration of primary tumor cells through different pathways to various locations after they fall off from the original malignant tumor, where they continue to grow and form a new secondary tumor of the same properties. In 1971, Folkman et al.[46] proposed a concept of the reliance of tumor growth and metastasis to angiogenesis. The tumor cell and inflammatory cells (primarily macrophage) in tumor tissues or the surroundings could produce factors with similar angiogenesis function, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). Some research reports claimed that VES inhibited angiogenesis in tumor by regulating expressions of VEGF, FGF, NF-B, Ras, and AP-1, which further prevented tumor growth and metastasis.[4,12,17,47] In addition, there was report that VES inhibited tumor metastasis by inhibiting matrix metalloproteinases-9 (MMP-9).[48]

Angiogenesis. VEGF is a positive control factor for angiogenesis and its bioactivities are regulated by endothelial tyrosine kinase receptor. VEGF makes up three receptors, which are structurally similar to tyrosine kinase: VEGF-R1, VEGF-R2, and VEGF-R3. In endothelium, VEGF-R2 is a primary sensor for VEGF signal. VES can possibly inhibit angiogenesis in tumor by inhibiting VEGF. A series of studies have proven this speculation. Malafa et al.[47] reported in 2000 that VES in a body could inhibit growth of an existing tumor. At the same time, VES could significantly inhibit the expression of VEGF in MDA-MB-231 tumor cells of breast cancer. In 2002, Malafa et al.[17] further reported that the evaluation of anti-tumor effect of VES by model of melanoma demonstrated VES could induce melanoma to enter a state of sleepiness and thus, inhibited the angiogenesis. Also, VES could significantly reduce the levels of VEGF, VEGF-R1, and VEGF-R2 proteins. It could inhibit the secretion of VEGF in melanoma and lowered the activities of VEGF promoter. These suggested that the inhibitory effect of VES on angiogenesis was possibly related to inhibition to transcription of VEGF gene. Fibroblast growth factors, FGF-1 and FGF-2, can promote proliferation and angiogenesis in malignant mesothelioma. Research showed that VES also inhibited proliferation of malignant mesothelioma by preventing expressions of FGF-1 and FGF-2, while this effect was not observed in non-malignant mesothelioma.[4,12] In 2004, Stapelberg et al.[4] discovered that high concentration of VES could induce apoptosis in malignant mesothelioma and low concentration of VES just inhibited its proliferation. This effect was not observed in non-malignant mesothelioma. The sub-apoptotic dosage of VES could induce selective down-regulation in expression of FGF-R1, while it was less effective to FGF-R2. FGF1 and FGF2 could promote proliferation of malignant mesothelioma, while VES could inhibit this effect. Also, hyperactive expression of a type of transcription factor of FGF-R1, E2F1, could weaken the inhibitory effect of VES on malignant mesothelioma. It was speculated that VES could selectively down-regulate expression of FGFR to inhibit proliferation of malignant mesothelioma. In 2005, Stapelberg et al.[12] found that VES could down-regulate the levels of FGF-1 and FGF-2 in malignant mesothelioma at the level of transcription and this effect was more profound for FGF-2. According to the EPR spectrum, it was speculated that the down-regulation of FGF-2 could be the result of decrease in transcriptional activities of egr1 after an elevation of active oxygen by VES was detected. Small interference RNAs (siRNA) of FGF-1, FGF-2, and egr1 could effectively inhibit proliferation of malignant mesothelioma, but the addition of extrinsic FGF-1 and FGF-2 weakened the effect. The reduction was more significant when FGF-2 was added, which could basically remove the inhibitory effect of siRNA. In addition, a similar compound to coenzyme Q, which targeted mitochondria and superoxide dismutase, could also minimize this inhibitory effect by VES on proliferation of malignant mesothelioma.

These data suggested that VES interefered with the autonomous secretion signal circuit of FGF–FGFR by producing active oxygen, which could further inhibit the proliferation. It further proved the speculation that VES inhibited angiogenesis in malignant mesothelioma by regulating FGF–1/–2. In 2007, Stapelberg et al.[49] showed in their newest research that VES destroyed the angiogenesis in malignant mesothelioma by inhibition of para–secreting signal of FGF–2.

Extracellular matrix. Infiltration and metastasis of tumor is a complex process, and extracellular matrix (ECM) is the first barrier to block tumor invasion. Tumor cells can secrete many enzymes to degrade extracellular matrix, such as matrix metalloproteinase (MMPs). Reduction in the activities of the matrix degradative system can inhibit infiltration and metastasis of tumor. VES has inhibitory effect on infiltration and metastasis of some tumors and the underlying mechanism may involve reduction of activities in MMP–9, which is secreted by tumor cells, in order to protect extracellular matrix. In an in vitro experiment on prostate cancer, after 24 hours of treatment with 20mol/L VES, the activities of MMP–9 in culture media of DU–145 and PC–3 tumor cells of prostate cancer were significantly lowered. And, it also inhibited the abilities of these two types of prostate cancer cells from penetrating Matrigel (a reconstruction matrix). As for prostate cancer cells, LNCaP, without secretion of MMP–9, its infiltration ability through Matrigel was low and it was not affected by VES. The research also demonstrated that VESs influence on PC–3 cell infiltration was not related to survival rate, cell cycle distribution, adhesiveness, and mobility. Its influence on the activities of secreted MMP–9 by PC–3 cells was also not related to gene expression at the level of RNA, neither was it related to inhibition on cell proliferation. 4B

Research on anti–tumor mechanism of VES and its prospect for application

In recent years, as people gain more knowledge in chemical prevention of tumor, we understand more about the anti–tumor mechanism of VES. Some researchers reported that the anti–tumor effect of VES was closely related to its unique molecular structure and could possibly be traced to its special domain. The anti–tumor effect of VES is achieved by several channels, and every link of the process occurs both intracellularly and extracellularly. This effect shows as inhibition on tumor growth, induction of cellular apoptosis, and inhibition on metastasis. VES can inhibit tumor growth by preventing DNA synthesis and postponing cell cycle. The regulation on regulatory proteins for cell cycle by VES can play an important role, even though this is still currently in debate and requires further investigation. Current school of thought believes that VES induces apoptosis in tumor cells by intrinsic and extrinsic pathways. The intrinsic pathway, which is mediated by mitochondria, is the primary approach for VES to induce apoptosis, while the non–mitochondrial extrinsic pathway, to some degrees, enhances the effect of the intrinsic one. Further in–depth research on intrinsic and extrinsic pathways may facilitate locating appropriate target site of medicine administration, as well as finding medicine with synergic effect to boost the anti–tumor effect of VES. There are still few studies on the inhibitory effect of VES to tumor metastasis. Some recent reports found that VES could inhibit metastasis by preventing angiogenesis in tumor. Clinical treatment for tumor metastasis is the most difficult problem to overcome and more research to unravel the inhibitory mechanism of VES on metastasis may be helpful for prevention and treatment of tumor.

Prior research proved that the anti–tumor effect of VES was superior to the traditional Vitamin E compound. However, pharmacokinetic data demonstrated that VES was quickly hydrolyzed into VE after absorption. Then, how did VES achieve the anti–tumor effect? Could there be an intermediate compound of VES? How much influence was its hydrolysis on the anti–tumor effect? How could we modify its dosage type to reduce the speed of its hydrolysis? The most recent report showed that VEBSA, which was VES in ether form, had a much more significant anti–tumor effect.[50] However, there was no other similar report and more studies should be conducted to improve the absorption rate and the bioavailability of VES. These studies to evaluate clinical application value of VES might promote oncological research to help people understand more of the progression mechanism of tumor, in order to find new target site for drug administration and even more effective and safe anti–tumor medicine.

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