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Vitamin K2 as a Chemotherapeutic Agent for Treating Ovarian Cancer

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1. Introduction

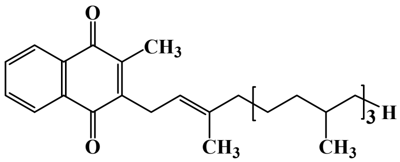
Ovarian cancer is the fifth most common cancer among women and approximately 200,000 women are diagnosed as ovarian cancer every year in the world (Parkin et al., 2005). Platinum-containing chemotherapeutic agents such as cisplatin (cis-diamminedichloroplatinum II) and carboplatin have been most frequently used for the treatment of ovarian cancer. These platinum compounds specifically react with guanine residues in DNA to form interstrand and intrastrand cross-links in DNA, known as DNA-adducts. This interferes with mitosis of cancer cells and causes cell death. However, ovarian cancer cells can become resistant to cisplatin and the majority of patients develop cisplatin-resistant disease (Giaccone 2000; Hennessy et al., 2009; Kartalou & Essigmann 2001), and as a result, most ovarian cancers relapse. Paclitaxel (taxol) and two analogues of camptothecin, irinotecan and topotecan, are the drugs most commonly administered to platinum-resistant ovarian cancer patients. Paclitaxel (taxol) binds to γ-tubulin and stabilizes microtubule structure, which causes a G2/M block in the cancer cell cycle. Irinotecan, also known as CPT-11 (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin) and topotecan (10-hydroxy-9-dimethylaminomethyl-(*S*)-camptothecin) inhibit topoisomerase I by resealing DNA breaks, which results in the inhibition of cancer cell growth (Bookman et al., 1998; Swisher et al., 1997). However, the survival rate for relapsed patients is low; Thus, there is an urgent need for more effective chemotherapeutic approaches for ovarian cancer treatment. We have previously reported that some ovarian cancer cell lines are remarkably sensitive to vitamin K2 (Shibayama-Imazu et al, 2003, 2006, 2008). In this review, strategies for developing chemotherapeutic agents for ovarian cancer are described and vitamin K2 is proposed as a promising chemotherapeutic agent for ovarian cancer.

2. Effect of vitamin K2 on cancer cells

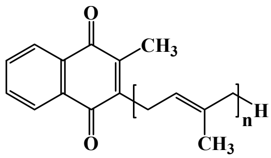
Natural forms of vitamin K such as vitamin K1 (phylloquinone) and vitamin K2 (menaquinones, MK) are cofactors for the post-translational γ-carboxylation of glutamate residues in vitamin K-dependent proteins (Fig. 1). Vitamin K is mainly used as a hemostatic agent because coagulation factors VII, IX, and X, which are critical to blood coagulation, are vitamin K-dependent proteins. In addition, vitamin K2 has anti-cancer activity, whereas vitamin K1 does not. This difference arises from structural differences in the side chains attached to the parent ring of vitamin K.

2.1 Structures and fundamental properties of vitamin K

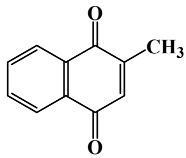
The parent ring structure of vitamin K is 2-methyl-1,4-naphthoquinone (menadione), which is also known as vitamin K3 (Fig. 1). Vitamin K3 does not occur naturally and causes oxidative stress in both normal and cancer cells and is toxic to the liver. There are few successful clinical applications of vitamin K3. Vitamin K1 has a phythyl side chain at the 3 position of vitamin K3 and is present mainly in green vegetables. Vitamin K1 is converted to vitamin K2 in animals and humans (Thijssen & Drittij-Reijnders, 1996); vitamin K2 has isoprenoid side chains of various lengths attached to the 3 position of the vitamin K3 ring structure. The different forms of vitamin K2, menaquinone-n (MK-n), are categorized according to the number of repeating isoprenoid residues in the side chain. The most common form of vitamin K2 in animals is menaquinone-4 (MK-4), which has four isoprenoid residues as its side chain. MK-4 is the most biological active form of the vitamin and is produced by intestinal bacteria. Long chain menaquinones, MK-7 to MK-10, are synthesized by bacteria and are present in fermented products such as cheese (MK-8 and MK-9) and East Asian fermented soybean products, such as natto and miso (Shearer et al., 1996; Schurgers & Vermeer, 2000).



Vitamin K1



Vitamin K2



Vitamin K3

Fig. 1. Chemical Structures of vitamin K

Naturally occurring vitamin K1 contains a phytyl group, and vitamin K2 has a repeating isoprenoid group at the 3 position of the vitamin K3 menadione ring. In animals, the most common and most biologically active form of vitamin K2 is MK-4, which has four isoprenoid residues ($n = 4$). Unlike vitamin K1, vitamin K2 has anti-cancer activity in addition to its critical role in blood coagulation and bone metabolism. The isoprenyl side chain of vitamin K2 contributes to its unique anti-cancer activity.

2.2 Growth inhibitory activity of vitamin K2 on cancer cells

We initially demonstrated vitamin K2-induced differentiation in human leukemia cells which was unrelated to its clinical role in blood coagulation and bone metabolism (Sakai I, 1994). Inhibition of cancerous cell growth and induction of apoptosis by vitamin K2 has subsequently been observed in a variety of human cancer cell lines, including liver (Nishikawa et al., 1995; Otsuka et al., 2004), pancreatic (Shibayama-Imazu et al., 2003), ovarian (Shibayama-Imazu et al., 2003, 2006, 2008), lung (Yoshida et al., 2003; Yokoyama et al., 2005), stomach (Tokita et al., 2006), breast (Wu et al., 1993), and leukocyte (Yaguchi, 1997). The growth-inhibitory effects of vitamin K2 on various human cancer cell lines are listed in Table 1. Vitamin K2 has almost no effect on normal bone marrow cells (Miyazawa et al., 2001), and inhibition of cancerous cell growth was not observed with vitamin K1. Therefore, the side chain of vitamin K2 may be important for the anti-cancer activity of vitamin K2. The anti-cancer activity of isoprenoids is known to depend on the length of the polyprenyl alcohol side chain; polyprenoids with a geranylgeranyl group or a geranylisopropyl group have the most potent anticancer activity (Ohizumi et al., 1995). Vitamin K2 MK-4 has a geranylgeranyl side chain and is commonly used for the treatment of a variety of cancer cells. In this review, vitamin K2 MK-4 is denoted simply as vitamin K2.

Source	Cell line	IC$_{50}$ (M)	Reference
Ovarian cancer	PA-1	5	Shibayama-Imazu et al., 2008
	TYK-nu	73	Shibayama-Imazu et al., 2008
	SK-OV-3	152	Shibayama-Imazu et al., 2008
	SW626	188	Shibayama-Imazu et al., 2008
	OVCAR3	>400	Shibayama-Imazu et al., 2008
Leukemia	U937	28	Shibayama-Imazu et al., 2003
	HL-60	150	Shibayama-Imazu et al., 2003
Liver cancer	PLC/PRF/5	>400	Shibayama-Imazu et al., 2003
	Hep2G	45	Otsuka et al., 2004
	Hep3B	112	Nishikawa et al., 1995
	HuH-7	80	Kanamori et al., 2007
Pancreatic cancer	KP-4	>400	Shibayama-Imazu et al., 2003
	MIA PaCa-2	153	Shibayama-Imazu et al., 2003
	2C6	>400	Shibayama-Imazu et al., 2003
Gastric cancer	KATO III	>400	Shibayama-Imazu et al., 2003
	NUGC-2	>400	Shibayama-Imazu et al., 2003
	MKN7	50	Tokita et al., 2006
	MKN74	25	Tokita et al., 2006
	FU97	35	Tokita et al., 2006
Lung cancer	LU-139	75	Yoshida et al., 2003

Table 1. IC$_{50}$ values for vitamin K2 in various cancer cell lines. IC$_{50}$ is defined as the concentration of vitamin K2 that inhibits cell growth by 50%. The values for SW626 and OVCAR3 were estimated from previously published results (Shibayama-Imazu et al., 2008). Clinical trials using vitamin K2 that were conducted successfully for leukemia and liver cancer patients are indicated by boxes.

3. Induction of apoptosis in ovarian cells by vitamin K2

Inhibition of cell growth is a good indicator of the induction of apoptosis in cancer cells by chemical agents. A comparison of the inhibition of the growth of various cancer cells by vitamin K2 showed that an ovarian cancer cell line PA-1 is the most sensitive to vitamin K2. A steroid orphan receptor TR3/Nur77, which regulates cell proliferation and apoptosis, is responsible for the induction of apoptosis in ovarian cancer PA-1 cells by vitamin K2.

3.1 Growth inhibition of ovarian cancer cells by vitamin K2

We observed that apoptosis is readily induced in some ovarian cancer cell lines by low concentrations of vitamin K2 (Shibayama-Imazu et al, 2003; 2006). Figure 2 shows that vitamin K2 is a potent inhibitor of the growth of human ovarian cancer PA-1 cells, with an IC_{50} of 5.0 $\pm$ 0.7 μM. The IC_{50} value for PA-1 cells is the lowest observed for cancer cell lines treated with vitamin K2, indicating that PA-1 cells are the most sensitive to vitamin K2 (Table 1). In contrast to PA-1 cells, SK-OV-3 cells were resistant to vitamin K2 and no significant growth inhibition was observed (Fig. 2). PA-1 and SK-OV-3 cells were used as vitamin K2-sensitive and vitamin K2-resistant cells, respectively, in order to examine the mechanism of apoptosis induction by vitamin K2.

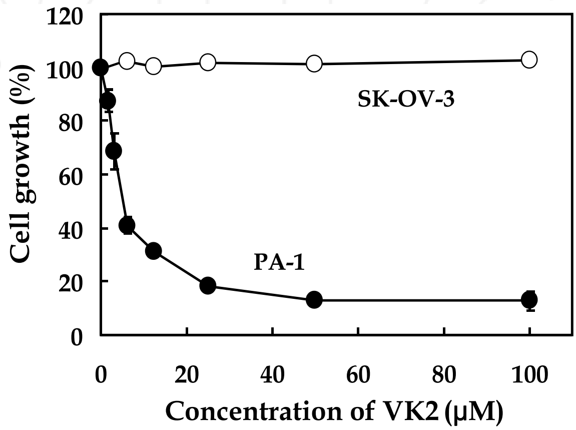


Fig. 2. Effects of vitamin K2 on the growth of human ovarian cancer PA-1 and SK-OV-3 cells. Cell proliferation was determined using the XTT assay 96 h after treatment with various concentrations of vitamin K2. Each value is represented as mean $\pm$ SD of the results from three independent experiments. Vitamin K2-sensitive PA-1(●); vitamin K2-resistant SK-OV-3 (○). [Reproduced with permission from Fig. 1 of Shibayama-Imazu et al., 2008.]

3.2 Mechanism of the induction of apoptosis by vitamin K2

The induction of apoptosis by vitamin K2 in vitamin K2-sensitive ovarian cancer PA-1 cells proceeds slowly. Fragmented nucelosomes were released into the cytosolic fraction 24 h after the start of incubation of PA-1 cells with 30 μM vitamin K2, and increased until at least 72 h after the start of incubation (Fig. 3A). After 72 h, the induction of apoptosis was evident in approximately 35% of PA-1 cells, as determined by counting apoptotic cells with condensed and fragmented nuclei stained with Hoechst 33342 (Shibayama-Imazu et al., 2008). The slow rate of apoptosis is one of the characteristic features of vitamin K2-induced apoptosis, compared to apoptosis induced by conventional anticancer agents such as camptothecin and etoposide, and by geranaylgeraniol (Masuda et al., 2000; Shibayama-Imazu et al., 2003). Mitochondria play a crucial role in the induction of apoptosis by various apoptotic agents. Cytochrome c released from mitochondria forms a complex with Apaf-1 and activates procaspase 9, which activates a downstream caspase cascade. Once the caspase cascade has been triggered, nucleases are activated to induce apoptotic chromatin condensation and DNA fragmentation. The release of cytochrome c in PA-1 cells was detected 48 h after treatment with 30 μM vitamin K2 and the release increased sharply 72 h after the treatment (Fig. 3B).

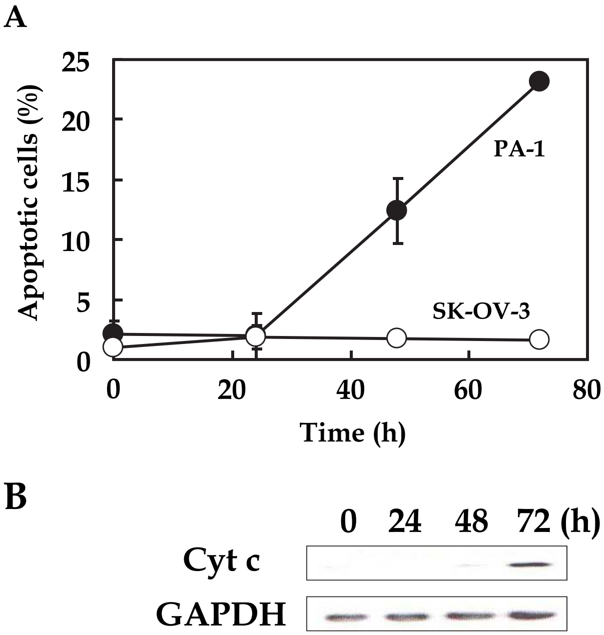


Fig. 3. Induction of apoptosis in ovarian cancer PA-1 and SK-OV-3 cells by vitamin K2. (A) Both vitamin K2-sensitive PA-1 (●) and vitamin K2-resistant SK-OV-3 (○) cells were treated with 30 μM of vitamin K2 for various times. Each value is represented as mean ± SD of the results from three independent experiments. The percentage of apoptotic cells that contained condensed and fragmented chromatin was quantified after staining with Hoechst 33342 as described previously (Shibayama-Imazu et al., 2008). (B) The panel shows immunoblotting analysis of cytochrome c released from mitochondria into the cytoplasm of PA-1 cells that had been treated with 30 μM vitamin K2 (Shibayama-Imazu et al., 2008). [Reproduced with permission from Fig. 6 of Shibayama-Imazu et al., 2008.]

3.3 Accumulation of TR3/Nur77 in mitochondria after treatment of PA-1 cells with vitamin K2

A steroid orphan receptor TR3/Nur77 is overexpressed in various cancer cells lines, including ovarian (Holmes et al., 2002), lung (Li et al., 1998), prostate (Li et al., 2000), colon (Cho et al., 2007), pancreatic (Chintharlapalli et al., 2005), bladder (Chintharlapalli et al., 2005) and stomach (Wu et al., 2002). The expression of TR3/Nur77 is rapidly induced during cancer cell apoptosis triggered by various apoptotic agents, such as phorbol ester 12-*O*-tetradecanoyl phobol-13-acetate (Li et al., 2000), etoposide (Li et al., 2000), cytosporone B (Liu et al., 2010), and the synthetic retinoid 6-[3-(1-admantyl)]-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437, Holmes et al., 2004). TR3/Nur77 translocates from the nucleus to mitochondria in response to these apoptosis inducers, with the exception of CD437 (Li et al., 2000). TR3/Nur77 binds to Bcl-2 which switches the function of Bcl-2 from protection to the induction of cytochrome c release from the mitochondria, resulting in the induction of apoptosis (Li et al., 2000). For CD437, the levels of TR3/Nur77 in the nuclei of various pancreatic cancer cells were increased by treatment with this agent, although translocation of TR3/Nur77 from the nuclei to the mitochondria was not observed (Chintharlapalli et al., 2005).

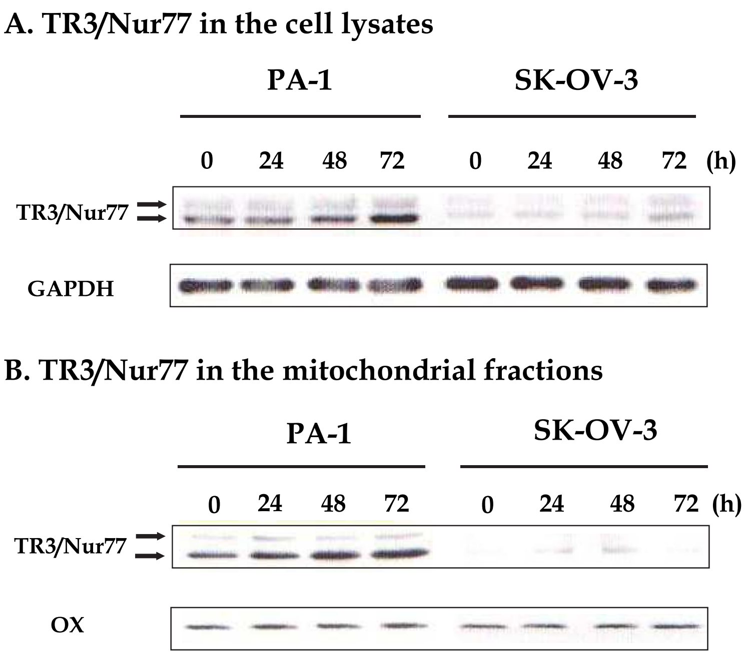


Fig. 4. Immunoblotting analysis of TR3/Nur77 in vitamin K2-sensitive PA-1 cells and vitamin K2-resistant SK-OV-3 cells. Both vitamin K2-sensitive PA-1 and vitamin K2-resistant SK-OV-3 cells were treated with 30 μM of vitamin K2 for 0 h, 24 h, 48 h, and 72 h. TR3/Nur77 in the cell lysates (A) and in the heavy mitochondrial fractions (B) were detected by immunoblotting using rabbit TR3/Nur77-specific polyclonal antibody (Shibayama-Imazu et al., 2008). Two immunostained bands were detected under our electrophoretic conditions as previously reported (Chintharlapalli et al., 2005). The more slowly migrating minor band is phosphorylated TR3/Nur77 (Pekarsky et al., 2001). The intensities of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) band and the cytochrome oxidase subunit IV (OX) band confirmed that an equal amount of cell lysate proteins and heavy mitochondrial fraction proteins, respectively, were loaded in each lane. [Reproduced with permission from Fig. 3 and Fig. 6 of Shibayama-Imazu et al., 2008.]

The level of TR3/Nur77 in vitamin K2-sensitive PA-1 cells was approximately four-fold higher than that in vitamin K2-resistant SK-OV-3 cells (Fig. 4A). The level of TR3/Nur77 in the cell lysate of PA-1 cells increased markedly in a time-dependent manner after treatment with vitamin K2. In contrast, the level of TR3/Nur77 in the cell lysate of vitamin K2-resistant SK-OV-3 cells was very low and was not significantly affected by treatment with vitamin K2. The TR3/Nur77 level in the cell lysate of SK-OV-3 cells did not reach the level observed in untreated PA-1 cells, even 72 h after the start of the treatment.

In the heavy mitochondrial fraction, which is composed mostly of mitochondria, the level of TR3/Nur77 increased sharply 48 h and 72 h after the start of the treatment of PA-1 cells with vitamin K2 (Fig. 4B). The percentage of apoptotic cells increased in parallel with the increase in TR3/Nur77 in the heavy mitochondrial fraction of PA-1 cells (Fig. 3A). In contrast, the level of TR3/Nur77 in the heavy mitochondrial fraction of vitamin K2-resistant SK-OV-3 cells was unchanged by vitamin K2 treatment (Fig. 4B). Immunofluorescence staining of PA-1 cells during vitamin K2-induced apoptosis indicates that the amounts of TR3/Nur77 present in both mitochondria and nuclei as well as in the cytosolic fraction increased after vitamin K2 treatment (Shibayama-Imazu et al., 2008). This suggests that TR3/Nur77 migrated directly from the cytoplasm to the mitochondria and no translocation from the nucleus to mitochondria occurred. It should be noted that this effect of vitamin K2 on ovarian cancer PA-1 cells is unique and different from that of apoptosis-inducing agents such as etoposide (Li et al., 2000) and cytosporone B (Liu et al., 2010) which cause translocation of TR3/Nur77 from the nuclei to the mitochondria.

4. Strategy for the improvement of chemotherapy of ovarian cancer

Combination treatments with agents that sensitize ovarian cancer cells to platinum compounds are an efficient strategy for overcoming chemoresistance acquired by ovarian cancer cells. Cancer cell survival signals can be inhibited by chemical agents, or ovarian cancer cells can be sensitized to platinum chemotherapeutic agents by preventing the repair of platinum-DNA adducts. A further method is to find chemical agents that induce apoptosis in ovarian cancer cells by a different mechanism from platinum compounds and may therefore exert synergistic apoptotic effects.

4.1 Inhibition of survival signals

The balance between cellular survival and induction of apoptosis determines the sensitivity of cancer cells to chemotherapeutic drugs. Therefore, blocking survival cascade signals or enhancing apoptosis-inducing signals enhances the sensitivity of ovarian cancer cells to chemotherapeutic agents (Vivanco & Sawyers, 2002). Topotecan, an inhibitor of topoisomerase I, inhibits Akt kinase activity in cisplatin-resistant ovarian cancer Caov-3 cells (Tsunetoh et al., 2010). The PI3K-Akt survival cascade signal in cisplatin-resistant ovarian cancer cells is inhibited by treatment with a combination of topotecan and cisplatin, which enhances the sensitivity to cisplatin *in vitro* and *in vivo* (Tsunetoh et al., 2010). The flavonoid compound, kaempherol, sensitizes ovarian cancer OVCAR-3 cells to cisplatin by down regulation of cMyc, which is involved in proliferation and is commonly activated in human cancer cells (Jung et al., 2008; Luo et al., 2010). Treatment of cisplatin resistant A2780CP ovarian cancer cells with a polyphenol, curcumin (diferulonyl methane), derived from the rhizomes of turmeric *Curcuma longa*, down regulated the expression of cMyc and pro-survival proteins such as Bcl-X_L and Mcl-1, leading to cisplatin sensitization (Yallapu, 2010). Topotecan, kaempherol, and curcumin therefore warrant further investigation as potential therapeutic agents for ovarian cancer.

4.2 Inhibition of platinum-DNA adduct repair

Inhibitors of DNA synthesis, such as gemcitabine (Touma et al., 2006), cytarabine (Swinnen et al., 2008), hydroxyurea (Raymond et al., 2001), and aphidicolin (Sargent et al., 1996), are able to inhibit the repair process of platinum-DNA adducts and have been used to increase sensitivity to chemotherapeutic platinum compounds. A phase II study using carboplatin followed by gemcitabine and paclitaxel for the treatment of ovarian cancer patients showed an improvement in therapeutic efficacy (Harries et al., 2004). However, the pulmonary toxicity observed as a side effect of this treatment still needs to be addressed.

The histone deacetylase inhibitor panobinostat, which affects the expression of various genes, showed synergistic cytotoxic effects in conjunction with conventional chemotherapeutic agents including carboplatin on ovarian cancer cell lines (Budman et al., 2010).

Arsenic trioxide inhibits UV-induced DNA repair processes (Hartwig et al., 1997), and also showed additive cytotoxic effects with cisplatin for human ovarian carcinoma cell lines *in vitro* (Uslu et al., 2000). Arsenic trioxide was successfully used for the treatment of all-trans retinoic acid resistant acute promyelocytic leukemia (Soignet et al., 1998), which suggests that it may be successful in treating cisplatin-resistant ovarian cancer patients.

4.3 Induction of apoptosis in cancer cells by activating the TR3/Nur77 gene

Indole -3-carbinol (I3C), contained in cruciferous vegetables such as broccoli, cabbage, and cauliflower, and its dimeric product 3,3′-diindolylmethane (DIM) are nontoxic, natural compounds with anticancer activities. DIM and its analogues increase the levels of TR3/Nur77 and induce apoptosis in various cancer cell lines including colon, pancreas, prostate, and breast cancer cells (Banerjee et al., 2009; Cho et al., 2007). Pancreatic cancer cells with acquired resistance to chemotherapeutic drugs, such as cisplatin, oxaliplatin, and gemcitabin, were sensitized by pretreatment with DIM (Banerjee et al., 2009). Compounds that activate the TR3/Nur77 gene and induce apoptosis in cancer cells are proposed as a new category of chemotherapeutic drugs, and include an analogue of cytosporone B (Liu et al., 2010) and 1,1-bis(3'-indolyl)-1-(*p*-substituted phenyl) methanes (C-substituted DIMs, Chintharlapalli et al., 2005). Acetylshikonin and its derivative 5,8-diacetoxyl-6-(1'-acetoxyl-4'-methyl-3'-pentenyl)-1,4-naphthaquinones (SK07) increased the level of TR3/Nur77 through posttranscriptional regulation, and induced apoptosis in various cancer cell lines including lung and cervical cancer cells (Liu et al., 2008). The positive correlation of the expression of the TR3/Nur77 subfamily member Nor-1 with survival rates of diffuse large B-cell lymphoma patients indicates the importance of TR3/Nur77 as a target for anti-cancer agents (Shipp et al., 2002). The importance of TR3-Nur77 as a therapeutic target of ovarian cancer is also demonstrated by the fact that low expression of TR3/Nur77 in tissue samples obtained from various cancer patients is significantly associated with metastasis of primary solid cancers (Ramaswamy et al., 2003).

We discovered that some ovarian cancer cell lines, such as PA-1 cells, are sensitive to vitamin K2 and apoptosis induced by stimulation of TR3/Nur77 synthesis and its accumulation in mitochondria (Fig. 5). Small interfering RNA (siRNA) directed against TR3-Nur77 (siRNA-TR3/Nur77) caused a marked decrease in the levels of TR3/Nur77 in the lysate of PA-1 cells. When ovarian cancer PA-1 cells after transfection with siRNA-TR3/Nur77 were treated with vitamin K2, the marked increase in the levels of TR3/Nur77 observed by vitamin K2 without siRNA-TR3/Nur77 was almost completely abolished (Shibayama-Imazu et al., 2008). Induction of apoptosis by vitamin K2 was also significantly inhibited by transfection of PA-1 cells with siRNA-TR3/Nur77. Furthermore, cycloheximide, an inhibitor of protein synthesis, prevented the increase in TR3/Nur77 levels, its accumulation in the mitochondria, and the induction of apoptosis in PA-1 cells caused by vitamin K2 treatment (Shibayama-Imazu et al., 2008). These results indicate that the synthesis of TR3/Nur77 and its accumulation in mitochondria are required for the induction of apoptosis in PA-1 cells by vitamin K2 and also suggest that an increase in the level of TR3/Nur77 could be the cause of sensitivity to the induction of apoptosis in PA-1 cells by vitamin K2 (Fig. 5).

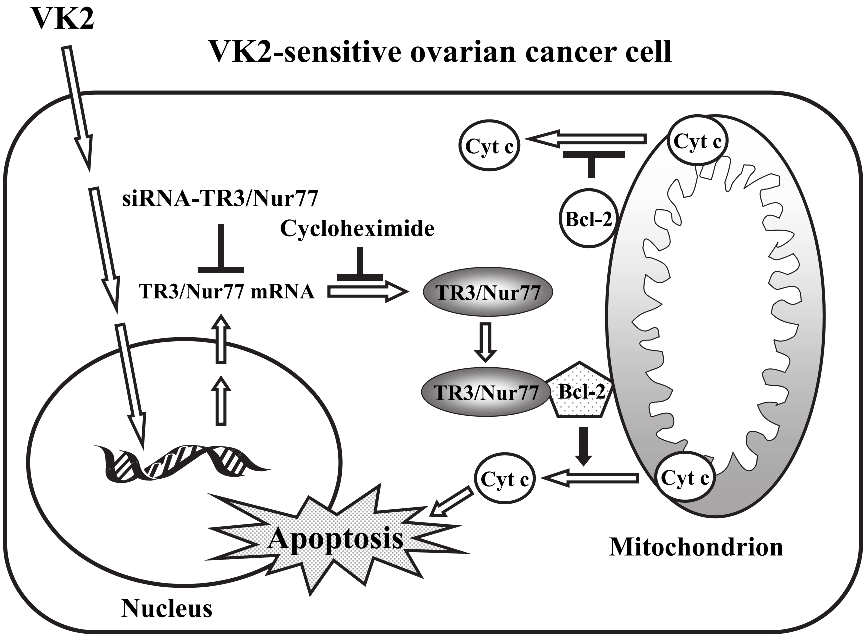


Fig. 5. A model for the induction of apoptosis in vitamin K2-sensitive ovarian cancer cells. The levels of TR3/Nur77 in vitamin K2-sensitive ovarian cancer cells are increased after treatment with vitamin K2. TR3/Nur77 synthesized in the cytoplasm migrates to the mitochondria and binds to Bcl-2, which protects the cell from apoptosis. Interaction of TR3/Nur77 with Bcl-2 induces a conformational change in Bcl-2, triggering the release of cytochrome c, which activates the caspase cascade and thus induces apoptosis. siRNA-TR3/Nur77 causes degradation of TR3/Nur77 mRNA and cycloheximide inhibits the protein synthesis of TR3/Nur77, both leading to inhibition of the induction of apoptosis by vitamin K2.

A combination of vitamin K2 and conventional chemotherapeutic agents for ovarian cancer, such as cisplatin and etoposide, which induce apoptosis by different mechanisms, might have additive or synergistic apoptotic effects on ovarian cancer cells. In addition, pretreatment with vitamin K2 or combination treatment with conventional chemotherapeutic agents could be effective for overcoming chemoresistance in ovarian cancer cells.

5. Vitamin K2 as a promising chemotherapeutic agent for ovarian cancer

Vitamin K2 induces apoptosis in blastic cells from patients with myelodysplastic syndrome (Nishimaki et al., 1999; Yaguchi et al., 1998) and has been successfully used in the clinical treatment of patients with this disorder (Miyazawa et al., 2000; Takami et al., 1999; Yaguchi et al., 1998, 1999). Oral administration of vitamin K2 with retinoic acid to a patient with relapsed acute promyelocytic leukemia resulted in complete remission (Fujita et al., 1998). In addition, oral administration of vitamin K2 also had a suppressive effect on the recurrence of hepatocellular carcinoma and improved patient survival rate (Habu et al., 2004; Mizuta et al., 2006; Tamori et al., 2007; Yoshiji et al., 2009). A recent cohort study indicates that cancer incidence and mortality were significantly decreased by dietary intake of vitamin K2 from food sources (Nimptsch et al., 2010). Vitamin K2 is also used clinically as a drug for osteoporosis in Asian countries such as Japan, Korea, and Thailand, because it also has a significant effect on bone fracture prevention (Cockayne et al., 2006; Olson, 2000; Shiraki et al., 2000). This is because calcium-binding proteins such as osteocalcin and calbindin, which are involved in calcium uptake and bone mineralization, are vitamin K-dependent proteins. A further cohort study indicated that the relative risk of coronary heart disease was also reduced by dietary intake of vitamin K2 (Geleijne et al., 2004). This may be because vitamin K-dependent proteins are associated with vascular repair processes (Benzakour & Kanthou, 2000) and the prevention of vascular calcification (Shanahan et al, 1998). No side effects from vitamin K2 therapy were observed in any of these clinical studies; even vitamin K2 dosages in excess of 40 mg/day did not cause any side effects associated with hypercoagulable states (Shiraki et al., 2000), demonstrating its excellent safety profile. However, it is unknown why some ovarian cancer cell lines are resistant to vitamin K2. It may be possible to overcome vitamin K2 resistance by using it in combination with other agents that increase the level of TR3/Nur77 in cancer cells, such as DIM, cytosporone B, and the shikonin derivative SK07. SK07 stimulates the protein synthesis of TR3/Nur77 even in cervical cancer HeLa cells, in which the basal expression level of TR3/Nur77 is low (Liu et al., 2008). Further pre-clinical and clinical evaluation of vitamin K2 is required for its use in chemotherapy for ovarian cancers.

6. Conclusion

Several ovarian cancer cell lines are sensitive to vitamin K2; the IC_{50} value of the most vitamin K2-sensitive ovarian cancer PA-1 cells is as low as 5 μM. Apoptosis is induced in PA-1 cells through the stimulation of TR3/Nur77 synthesis and its accumulation in mitochondria, which results in the release of cytochrome c and activation of the caspase cascade (Fig. 5). Because this mechanism is different from those of conventional chemotherapeutic agents for ovarian cancer such as cisplatin and etoposide, the present study demonstrates a new method for increasing the sensitivity of cisplatin resistant ovarian cancer cells to chemotherapy. Moreover, our observation suggests that the combination of vitamin K2 with cisplatin or etoposide may potentially be effective for the treatment of ovarian cancers. None of the clinical trials using high doses of vitamin K2 have recorded side effects from the treatment, and oral administration of vitamin K2 has already been successfully used for the treatment of acute promyelocytic leukemia and haepatocellular carcinoma (Table 1). We therefore propose vitamin K2 as a useful chemotherapeutic agent for ovarian cancer.

7. Acknowledgements

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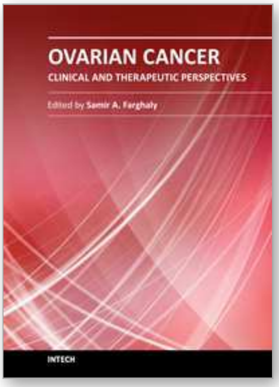

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Worldwide, Ovarian carcinoma continues to be responsible for more deaths than all other gynecologic malignancies combined. International leaders in the field address the critical biologic and basic science issues relevant to the disease. The book details the molecular biological aspects of ovarian cancer. It provides molecular biology techniques of understanding this cancer. The techniques are designed to determine tumor genetics, expression, and protein function, and to elucidate the genetic mechanisms by which gene and immunotherapies may be perfected. It provides an analysis of current research into aspects of malignant transformation, growth control, and metastasis. A comprehensive spectrum of topics is covered providing up to date information on scientific discoveries and management considerations.

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