development in TRAP rats is very sensitive to chemicals that modulate the AR axis, including the endogenous androgen level. The possibility that suppressive effects of ARBs were due to down-regulation of the transgene expression could be excluded by the data on SV40 T antigen expression shown in Figures 2A and S4A. The in vivo finding that ARBs not only down-regulated AR protein but also suppressed the androgen responsive gene, GK11, an ortholog of human PSA, at the mRNA level, provided evidence that ARBs functionally suppressed the AR pathway in prostatic lesions of TRAP rats. Thus, the present study highlighted that the main pathway responsible for attenuation of prostate carcinogenesis by ARBs is the AR signal pathway through suppression of AR-mediated transcriptional activity by both AR down-regulation and ERβ up-regulation, as well as inactivation of the p38 MAPK pathway.

ERβ is known to regulate prostate gland growth as an antiproliferative receptor [31]. This study demonstrated that ERβ is one of the downstream molecules of AT1R, and the ERβ signal transduction pathway plays an important role in the mechanisms of suppression of prostate carcinogenesis by ARBs. Genistein and deizein, major components of soybean isoflavone, have been shown to exert suppressive effects on rat prostate carcinogenesis [32], and these compounds are known to bind ERβ and to have ERβ agonistic activity [33]. Gamma-tocopherol has also demonstrated an inhibitory effect on prostate carcinogenesis in TRAP rats by activation of caspase signaling [15] and it is speculated that the ERβ signal pathway might be involved in these inhibitory effects because gamma-tocotrienol was recently revealed to induce apoptosis by activation of caspase 3 and ERβ signaling [34]. The latest report suggests that ERβ exerts a pivotal role in sustaining the epithelial phenotype and suppressing the acquisition of epithelial-mesenchymal transition and aggressive characteristics of prostate cancer [35]. This accumulating evidence suggests that modulators of ERβ might be potential chemopreventive or chemotherapeutic agents.

Among the ARBs used here, telmisartan demonstrated PPARγ activation while candesartan and olmesartan did not [36]. Additionally, olmesartan is known to increase angiotensin 1–7 levels through activation of angiotensin converting enzyme 2 [37]. No toxic effects were observed in TRAP rats treated with candesartan while significant suppression of body weight gain was found with the high-dose telmisartan. These phenomena are presumably PPARγ-related and similar effects of telmisartan have been reported previously [38,39]. However, it has been proven to also potentiate the signaling of PPARα or PPARδ in mice [40,41], and PPARδ activation is deeply involved in the prevention of body weight gain by telmisartan [42].

Androgen deprivation therapy remains the gold standard first-line treatment for prostate cancer; however, most tumors gradually acquire a castration-resistant phenotype. Several signal pathways responsible for the pathogenesis of CRPC have been elucidated, and growth and survival of CRPC continue to depend on a functional AR signal pathway that is adapted to a microenvironment of low androgen levels [43,44]. At present, several agents targeting AR signaling have been developed, such as a new type of anti-androgens, CYP17 inhibitors, HSP90 inhibitors, histone deacetylase inhibitors, and tyrosine kinase inhibitors [45]. The present data suggest that ARBs are also candidates for suppressor drugs of the AR signal pathway by attenuating AR-mediated transcriptional activity.

Our previous report indicated that ARBs have the potential to decrease or stabilize PSA level of patients with CRPC and inhibit the occurrence of symptoms such as bone pain [9]. These effects seemed to be due to the anti-inflammatory and anti-angiogenesis activity of ARB. In this study, we examined whether ARBs could clinically affect the growth of hormone-naïve cancer cells. In general, it has been reported that aPSA-DT was longer than ePSA-DT from the nadir of biochemical failure (BCF), PSA 0.2 ng/ml [46]. However, olmesartan treatment markedly prolonged PSA-DT in patients with BCF, that is, PSA >0.2 ng/ml after RP, in comparison with non-treated patients (control patients). It is well known that PSA-DT after RP is strongly associated with the risk of cause-specific mortality [47], and is a predictor of development of metastasis [48–50]. In the present study, administration of olmesartan prolonged aPSA-DT 2 fold compared with non-treated patients. These clinical data are consistent with in vivo and in vitro data showing that ARBs have the property to suppress the progression of prostate cancer associated with PSA decrease.

Interspecies scaling is commonly used to extrapolate doses from animal experiments to humans. It is known that plasma concentrations are better than dose levels for the interpretation of animal studies. An approximately five times higher dose in rats compared with human dose level is necessary to reach similar average steady-state plasma levels [51]. Moreover, the 0.75 power of body weight method is conventionally used in scaling [52,53]. The formula is $(\text{mg dose in rat})/(\text{rat body weight})^{3/4} = (\text{mg dose in human})/(\text{human body weight})^{3/4}$, and this can be rewritten as $(\text{mg/kg/day dose in rat}) \times (\text{rat body weight})^{1/4} = (\text{mg/kg/day dose in human}) \times (\text{human body weight})^{1/4}$. Applying this formula in the present

experiments using TRAP rats, an equivalent dose of ARBs in human (mg/kg/day) = (10 mg/kg/day) × $(0.3\ kg)^{1/4}/(70\ kg)^{1/4}$ = 2.56 mg/kg/day. This value is equivalent to an intake 2–4 times higher for telmisartan or 14–22 times higher for candesartan than the respective standard intake level in a 70 kg-sized person. In practical terms, however, candesartan is effective for suppressing serum PSA levels at a dose within 4–8 mg/day in advanced prostate cancer patients [9,10]. Therefore, a normal dose of ARBs used in the clinical scenario should provide satisfactory responses of human prostate cancers.

In conclusion, ARBs impede prostate cancer progression by affecting AR expression. These data may contribute to the establishment of a novel chemopreventive and alternative chemotherapeutic strategy for human prostate cancer.

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Cancer chemoprevention through the induction of apoptosis by natural compounds*

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ABSTRACT

As cell and tissue homeostasis are mediated by the balance between proliferation and apoptosis, controlling this balance is important for cancer chemoprevention. Cancer chemoprevention can be achieved by the use of natural, synthetic or biologic compounds that reverse, suppress or prevent the development of epithelial malignancies. Natural compounds including flavonoids are able to reduce oxidative stress, which is the most likely mechanism mediating the protective effects against cancer development. In addition, *in vitro* and *in vivo* studies have suggested that flavonoids, such as (-)-epigallocatechin-3-gallete (EGCG), quercetin, and curcumin, act by induction of apoptosis. Several natural compounds inhibit cell proliferation and angiogenesis. Certain natural products have been shown to inhibit the activation of nuclear factor kappa B (NF-κB) and Akt signaling pathways, both of which are known to maintain a homeostatic balance between cell survival and apoptosis. Understanding the mechanism of these natural products will contribute to the development of more specific preventive strategies against cancer development. Here we focus on the ability of natural cancer chemopreventive agents to induce apoptosis, and attempt to provide evidence for the preventive and therapeutic effects of natural compounds, EGCG, quercetin, and curcumin, in a succinct manner highlighting κand Akt signaling pathways *in vivo*.

Keywords: Cancer Chemoprevention; Apoptosis; Natural Compounds

1. INTRODUCTION

Epidemiological studies have shown that diet consisting of a high rich in fruit and vegetable reduces the risk of several types of cancer [1]. Intake of fruit and vegetables has been successfully used in the prevention of chronic diseases associated with oxidative stress conditions, including cancer [2,3]. The cancer preventing properties of fruit and vegetables have been ascribed, at least in part, to their high content of polyphenols [4]. The majority of polyphenols present in food are flavonoids and phenolic acids that are an integral part of the human diet. Laboratory rodent studies have shown that polyphenols have cancer-preventing properties and considered to be potential chemopreventive agents [5-7]. They can influence important cellular and molecular mechanisms associated with multiple carcinogenic steps, such as expression of key proteins in signal transduction pathways (e.g., mitogen activated protein kinases (MAPKs) or activator protein (AP)-1), the transcription factor nuclear factor-kappa B (NF-κB) and its its downstream gene products, modulation of cell-cycle regulation and induction of apoptosis [7], which affect cell differentiation, proliferation and apoptosis, immune responses and metabolism of carcinogens [4].

Apoptosis is conceivably the most potent defense against cancer development since it is the mechanism used by metazoans to eliminate deleterious cells. Many chemopreventive agents have been shown to induce apoptosis in transformed cells both *in vitro* and *in vivo*. Induction of apoptosis appears to be associated with their effectiveness in modulating carcinogenic processes [8]. Since apoptosis provides a physiologic mechanism for eliminating initiated or abnormal cells, dietary factors affecting apoptosis can influence carcinogenesis. In fact, activation of apoptosis in pre-cancerous cells is one of the most important mechanisms of cancer chemoprevention by dietary factors [9].

In this review, we focus on the apoptosis-inducing properties of several natural compounds present in the human diet and describe their beneficial effects against cancer development. Understanding the mechanism of these natural products will contribute to the development

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of more specific preventive strategies against cancer.

2. CHEMOPREVENTIVE COMPOUNDS IN FOOD

An effective chemopreventive agent should preferably intervene early in the process of carcinogenesis to eliminate pre-malignant cells before they acquire malignant character. Many chemopreventive agents are able to block or delay the promotion and/or progression of pre-malignant or malignant cells by modulating cell proliferation and/or differentiation [8,10] and, therefore, should be chronically administered to individuals with a higher risk of cancer development. However, using this approach, even minor adverse effects would be unacceptable: obstacles to the use of chemoprevention for many cancers include long-term toxicity and the development of chemoresistance [8]. These issues can limit the feasibility and success of conventional forms of chemoprevention for many cancers. Alternative approaches involve the use of agents that eliminate cells expeditiously. Targeted delivery of apoptosis-inducing agents to tumor cells would also prevent the need for chronic exposure, limiting the risk of long term toxicity and/or the development of chemoresistance [8].

An ideal chemopreventive agent should be selective for damaged or transformed cells, display a significant bioavailability in the target lesion and have more than one mechanism of action. Moreover, it should be highly effective, easy to administer, and inexpensive. Dietary compounds are particularly attractive because of long-standing exposure to them by humans, their relative lack of toxicity, and encouraging indications from epidemiological studies [11]. Indeed, numerous dietary compounds and micronutrients are emerging that have considerable potential for hindering *in vivo* deleterious oxidative processes and inducing apoptosis in cancer cells. One potentially important drawback of dietary compounds is their possible low bioavailability after ingestion [12,13].

Food contains several promising chemopreventive compounds [14-16]. Plant polyphenols, ubiquitous in a diet rich in fruit and vegetables, are thought to be responsible for the cancer protective effects ascribed to this type of diet [4,5,13]. In fact, numerous phenolic compounds have been shown to display anti-proliferative and cytotoxic effects towards several tumor cells, presenting toxic effects that specifically target cancer cells rather than normal cells [17-20]. Dietary polyphenols are predominantly consumed through fruit and beverages (juice, wine, tea, coffee, chocolate and beer), with the exception of vegetables, cereals and olive derivatives that are mainly associated with the Mediterranean diet [21-23]. Their average daily intake has been reported to be approximately 1 g [13,24], which is much greater than the intake of all other classes of dietary anti-oxidants. For example, this value is approximately 10-times higher than vitamin C intake and 100-times greater than the intake of both vitamin E and carotenoids [24].

In addition to the anti-oxidant properties, polyphenols exert interesting biological abilities in animal experiments and *in vitro* systems. The compounds are able to trap and scavenge free radicals, decrease leukocyte immobilization, induce apoptosis, inhibit cell proliferation and angiogenesis, and exhibit phytoestrogenic activity [25-27]. Dietary polyphenols interfere with signal transduction pathways related to the carcinogenesis process, thereby acting as chemopreventive agents. They include the suppression of NF-κB and activating protein (AP-1) activation, inhibition of the mitogen-activated proteins (MAPKs)-, protein kinases- and growth-factor receptor-mediated pathways, cell cycle arrest, induction of apoptosis, anti-oxidant and anti-inflammatory effects, and suppression of angiogenesis [7].

The chemopreventive properties of a variety of compounds can be directly related to their pro-apoptotic properties, most probably exerted via the intrinsic (mitochondrial) apoptotic pathway [8,10] (**Table 1**).

Pro-apoptotic diet-derived compounds can conceivably protect against cancer by enhancing elimination of initiated, precancerous cells. Polyphenols have been shown to induce pro-apoptotic responses in malignant or pre-malignant cells. Many studies have described the pro-apoptotic properties of dietary polyphenols in a varietry of human cancer cell lines derived from colon, prostate, lung, breast cancers, and leukemia [28]. A major catechin, EGCG, among green tea catechins is the most potent polyphenolic compound with respect to inducing apoptosis and inhibiting proliferation in cancer cells: it induces apoptosis in and suppresses growth of human cancer cell lines, such as breast adenocarcinoma (MCF-7 and MDA-MB-231 cells) [29], oral squamous carcinoma [30], leukemia [31], breast [32], lung [33], prostate [34] and colon [35] cancers.

Several studies have indicated that phenolic concentrations leading to apoptosis are within the micromolar range. For example, quercetin reportedly induces apoptosis at concentrations ranging from 29 M to 150 M [36,37]. In general, the effective concentrations required for induction of apoptosis are higher than those leading to growth inhibition. However, some studies have reported the induction of apoptosis without inhibition of cell proliferation (e.g. EGCG effect on H661 lung cancer cells) [38]. Further increases in polyphenol concentration may cause necrosis of all cell lines tested [39].

Recent studies have proposed that different dietary phenolic compounds may act synergistically, together with established anti-cancer agents and act as enhancers of anti-carcer drugs [40,41] (**Table 2**). For example, the synergistic effects of EGCG and curcumin in pre-ma-

lignant and malignant human oral epithelial cells [42], resveratrol and quercetin in human pancreatic cancer cells [36], quercetin and cisplatin in human laryngeal Hep2 cells [43] and HeLa cells [44], and EGCG and sulindac or tamoxifen against human PC-9 lung cancer cells [45] have been described. This recognized synergy among dietary phenolics and conventional synthetic drugs provides an interesting approach to combination therapy as well as for the pre-treatment of neoplastic cells with polyphenolic agents. This strategy has been able, in some cases, to even overcome chemoresistance [46].

The anti-carcinogenic and cytotoxic activities of polyphenols are largely determined by structural parameters, as much as their anti-oxidant potencies [20,47-50]. Despite their close resemblance, their bioactivity varies considerably upon minor structural modifications, since such modifications often induce significant conformational changes [7,51,52]. This implies an important drawback in the understanding of the effects of polyphenols in human health, when considering the huge number of different compounds (>8000) [12].

3. APOPTOSIS

Although the term apoptosis was first used in 1972 [53] to describe a morphologically distinct form of cell death, several components of this concept have only recently been described in detail. The process of programmed cell death is characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. Apoptotic cells show morphological changes such as cell shrinkage, pyknosis and extensive plasma membrane blebbing leading to the formation of apoptotic bodies, which are subsequently phagocytosed by macrophages,

Table 1. Anticancer effect and mechanisms of natural compounds through NF-κB or PI3K/Akt pathway *in vivo*.

Compounds	Plant	Target organs	Carcinogens/cell line	Animal	Effects/molecular targets	Ref No.
(-)-Epigallocatechin gallete (EGCG)	Green tea	Intestine	None	*Apc*$^{Min+/-}$ mice	Attenuates aberrant nuclear β-catenin and activated Akt and ERK signaling	115
		Intestine	Azoxymethane	c57bl/ksj-*db/db* mice	Overcomes the activation of the IGF/IGF-IR axis, improving hyperlipidemia, hyperinsulinemia, and hyperleptinemia	116
		Liver	Diethylnitrosamine	c57bl/ksj-*db/db* mice	Inhibits IGF/IGF-1R axis, improving hyperinsulinemia, and attenuating chronic inflammation	117
		Tumor-associated endothelial cells and endothelial progenitor cells	A375SM (melanoma), xenograft	Nude mice	Selective anti-angiogenic effects, inhibits the phosphorylation of Akt in tumor-associated endothelial cells, MMP-9 mRNA expression level and vascular endothelial growth factor in endothelial progenitor cells	118
		Urinary bladder	UM-UC-3, xenograft	Nude mice	Down-regulates N-cadherin and inactivation of Akt signaling	119
Curcumin	Curcuma longa	Head and neck	CAL27 (squamous cell carcinoma), xenograft	Nude mice	Suppresses the activation of NF-κB without affecting the expression of pAKT	124
		Breast	MDA-MB23 (adenocarcinoma), xenograft	Nude mice	Inhibits survivin, NF-κB and its downstream effectors cyclin D1 and Bcl-2, and strongly up-regulated p21WAF1	125
		Head and neck	SCC40 (squamous cell carcinoma), xenograft	Nude mice	Blocks nicotine-induced activation of the AKT/MTOR pathway in HNSCC, which retards cell migration, reduced MMP-9 expression	128
			4-Nitroquinoline 1-oxide	CBA/CaJ mice		
		Colon	HCT-116 (adenocarcinoma), xenograft	Nude mice	Decreases COX-2, IL-8, and VEGF mRNA and protein expression, decreased AKT and extracellular signal-regulated kinase activation	133
		Brain	B16F10 (mouse melanoma), xenograft	c57bl mice	Suppresses Cyclin D1, p-NF-κB, BclXL, p-Akt, and VEGF	134
Quecetin	Vegetable and fruits	Salivary gland	ACC-2 and ACC-M (adenoid cystic carcinoma), xenograft	Nude mice	Down-regulates the PI3K/Akt/IKK-α/NF-κB signaling pathway	147

Table 2. Synergistic induction of apoptosis by combining natural compounds and anticancer drugs/radiation through NF-κB or PI3K/Akt pathway *in vivo*.

Compounds	Combination agent	Target organ	Carcinogen/cell line	Animal	Effects/molecular targets	Ref. No.
EGCG	Tamoxifen	Breast	Xenograft, MDA-MB-231 (estrogen receptor-negative breast cancer)	Nude mice	Decreases the tumor protein expression of mTOR and decreases of the expression of EGFR, NF-κB, b-Raf, p-MEK, S6K, 4EBP1, Akt, vascular EGFR-1 (VEGFR-1) and VEGF	148
Quercetin	Sulforaphane	Pancreas	Xenograft, MIA-PaCa2 (pancreatic cancer)	Nude mice	Affects the self-renewal potential, ALDH1activity, apoptosis induction, inhibition of angiogenesis, NF-κB and epithelial-mesenchymal transition processes	153
	trans-Pterostilbene (trans-3, 5-dimethoxy-4'-hydroxystilbene, t-PTER),FOLFOX6, radiation	Colo-rectum	Xenograft, HT-29 (colorectal cancer)	Nude mice	Over-expresses superoxide dismutase 2 and down-regulates of bcl-2 expression by inhibiting NF-κB activation	155
Curcumin	Radiation	Colo-rectum	Xenograft, HCT-116 (colorectal cancer)	Nude mice	Potentiates the anti-tumor effects of adiation therapy by suppressing NF-κB and NF-κB-regulated gene products, leading to inhibition of proliferation and angiogenesis	123
	EGCG	Breast	Xenograft, MDA-MB-231 (breast cancer)	Nude mice	Decreases the level of VEGFR-1 protein expression, decreases the tumor protein levels of EGFR and Akt	150
	Gemcitabine	Pancreas	Xenograft, MIA PaCa2 (pancreas cancer)	Nude mice	Potentiates the anti-tumor effects of gemcitabine by suppressing proliferation, angiogenesis, NF-κB, and NF-κB-regulated gene products	151
			Xenograft, Pa03C (pancreas cancer)	Nude mice	Reduces the activation of NF-κB as well as the expression of matrix metalloproteinase-9 and cyclin D1	152
	resveratrol	Prostate	None	Prostate-specific PTEN knockout mice	Negatively regulates of the activated p-Akt, cyclin D1, AR and mTOR	154
	Paclitaxel	Uterine cervix	Xenograft, HeLa cells (uterine cervical cancer), 3-methylcholanthrene	NOD-SCID mice	Augments the anti-tumor action of paclitaxel by down-regulating the activation and down-stream signaling of anti-apoptotic factors and survival signals such as NF-κB, Akt and mitogen-activated protein kinases	156
	Cisplatin	Head and neck	Xenograft, CAL27 (SCC)	Nude mice	Inhibites cytoplasmic and nuclear IKKβ, resulting in inhibition of NF-κB activity	157
	Dasatinib	Colo-rectum	None	$Apc^{Min+/-}$ mice	Suppresses EFGRs, IGF-R and c-Src signaling pathway, decreases the activation of down-stream signaling pathways, Akt and Erk(s), associated with decreased NF-κB activity	158

parenchymal or neoplastic cells. Apoptosis rarely causes an inflammatory response, since 1) apoptotic cells do not release their constituents into the surrounding interstitial tissue, 2) apoptotic cells are quickly phagocytosed by cells in the surrounding tissue, preventing secondary necrosis, and 3) the engulfing cells do not produce inflammatory cytokines [54].

Apoptosis can be initiated by receiving extracellular or intracellular signals including growth factor withdrawal, UV- or gamma-irradiation, chemotherapeutic agents, heat shock, nutrient deprivation, and by a family of transmembrane proteins called death receptors. These signals are transduced to adapter proteins and transmitted to specific cysteine proteases called "initiator caspases". At this point the cell is committed to undergo apoptosis, followed by "execution of cells" (mediated by sequential activetion of the so-called "executioner caspases"), systematic disintegration of cell structure and phagocytosis of the cell corpses [55].

Caspases (cysteine-dependent aspartate-specific prot-

eases) are typically activated iduring the early stages of apoptosis. This family of proteins is synthesized as inactive zymogens but, once activated, can begin a proteolytic cascade, which results in the cleavage of key cellular components required for normal cellular function, including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. Caspases can also activate other degradative enzymes such as DNAases, which begin to cleave the DNA in the nucleus. To date, 14 different members of the caspase-family (**Table 3**) have been described in mammals [55-57]. The ten major pro-apoptotic caspases can be classified as initiators (caspase-2, -8, -9, -10), effectors or executioners (caspase-3, -6, -7) and inflammatory caspases (caspase-1, -4, -5) [55,58]. Other caspases that have been identified to date (caspase-11, -12, -13 and -14) are involved in specific apoptotic processes or expressed solely in specific types of tissue [55].

It is currently accepted that apoptosis can occur via two main pathways: the extrinsic or death receptor (**Figure 1**) and intrinsic or mitochondrial (**Figure 2**) pathways [59]. The extrinsic pathway initiated extracellularly via activation of cell surface receptors by specific molecules known as pro-apoptotic ligands including CD95L/FasL (receptor CD95/FasR), and Apo2L/TRAIL (receptors DR4, DR5) [53]. The Apo2 ligand has sparked growing interest within the oncology field due to its reported ability to selectively trigger cancer cell death [60,61]. Once activated, the death domains of these receptors, bind to the adaptor protein Fas-associated death domain (FADD), resulting in the assembly of the death-inducing signaling complex (DISC), and recruitment and assembly of the initiator caspase-8 and -10 [62]. Once activated, caspase-8 directly activates caspase-3 to initiate degradation of the cell. Active caspase-8 can also cleave Bid (pro-apoptotic) to tBid, which binds to the mitochondrial membrane to facilitate the release of cytochrome c and initiate the intrinsic pathways. This allows "cross-talk" between the two main pathways and amplifies the apoptotic signaling from death receptors.

The extrinsic and intrinsic pathways both end at the point of the execution phase. Execution caspases activate a cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade nuclear and cytoskeletal proteins. Such executioners including caspase-3 (considered to be the most important executioner caspase) can be activated by any of the initiator caspases (caspase-8, -9 or -10).

In addition to p53, the extrinsic and intrinsic pathways are also regulated by NF-κB, the ubiquitin proteosome system and the phosphatidylinositol-3-kinase (PI3K) pathway [63]. NF-κB is one of the most studied transcription factors in mammalian cells. Its function has been implicated in inflammation, cell proliferation, differentiation, apoptosis, cell survival and tumorigenesis [64]. NF-κB describes a ubiquitously expressed family of five proteins: p65 (RelA), p50, p52, c-Rel and RelB. Many stimuli produce survival responses in cells that are mediated by NF-κB. Indeed, overall reduction in NF-κB activity has been associated with an increased apoptotic index in many cell types [65]. Inactive NF-κB is bound to IKB inhibitory proteins. Once activated, NF-κB exhibits both

Table 3. Caspases[a] involved in apoptosis or programmed cell death.

Type	Name	Synonyms
Initiator or apical	Caspase-2	ICH1, Nedd2
	Caspase-8	FLICE, MACH1, MCH5, FADD-like Ice
	Caspase-9	MCH6, ICELAP6
	Caspase-10	FLICE2, MCH4
Effectors or executioner	Caspase -3	CPP32, YAMA
	Caspase -6	MCH2
	Caspase -7	MCH3, CMH, ICELAP3
Inflammatory	Caspase -1	ICE
	Caspase -4	ICH2, TX, ICErII
	Caspase -5	ICErIII, TY
	Caspase -11	-
	Caspase -12	-
	Caspase -13	ERICE
	Caspase -14	MICE

a: Caspase = cysteinyl aspartic acid-protease.

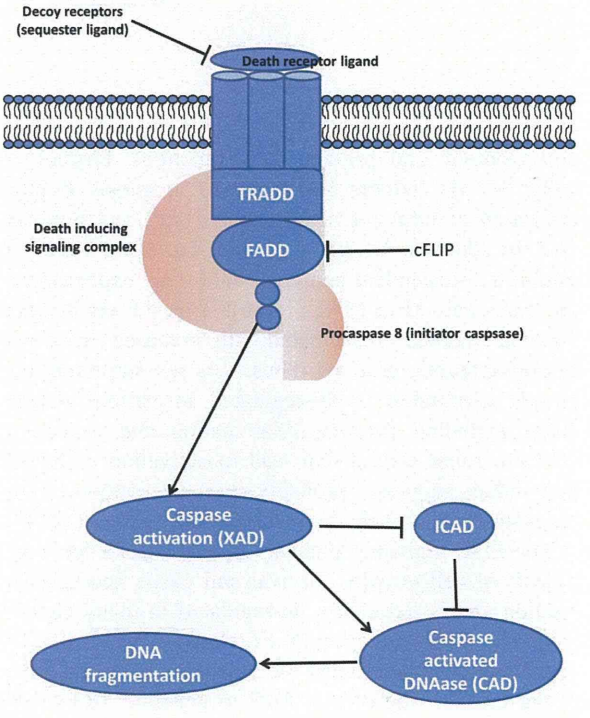

Figure 1. Extrinsic pathway.

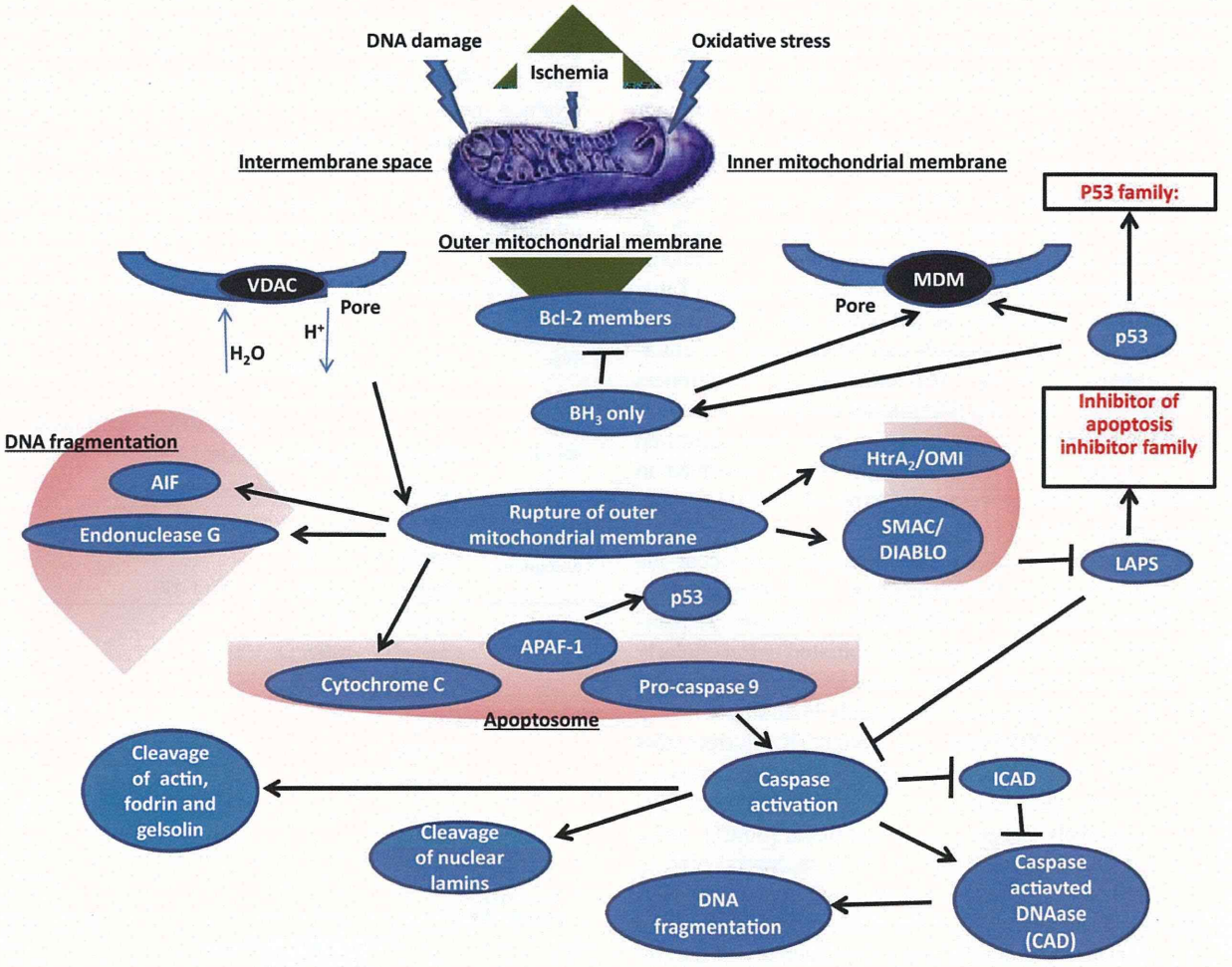

Figure 2. Intrinsic pathway.

anti-apoptotic and pro-apoptotic functions. Physiologically, NF-κB induces resistance to apoptosis through activation of inhibitor of apoptosis (IAP) and X-linked IAP. In addition, NF-κB activation has been shown to inhibit p53-dependent apoptosis following expression of the oncogene AP12/MALT1 [66]. This NF-κB directed survival response is associated with increased expression of anti-apoptotic proteins. Thus, it is not surprising that NF-κB expression is dysregulated in various disease states including chronic inflammation and cancer. In contrast, some stimuli that lead to activation of NF-κB may induce apoptosis, probably via the activation of pro-apoptotic proteins such as c-myc, p53 and caspase-1 [67].

The PI3K signaling pathway is also crucial for many aspects of cell growth, survival and tissue neo-vascularization and is frequently up-regulated in many cancers [68]. The PI3Ks are a family of related enzymes that are capable of phosphorylating the 3 position hydroxyl group of the inositol ring of PI(4,5)P2 to generate PI(3,4,5)P3 [69]. Upon activation of the PI3K pathway by many growth factors such as epidermal growth factor (EGF), PI(3,4,5) P3 is produced on the inner side of the plasma membrane and binds to Akt. Akt inactivates pro-apoptotic factors such as Bad, which controls the release of cytochrome c [70,71], procaspase-9 and Forkhead transcription factors (such as FOXO). Akt also activates anti-apoptotic genes including the cyclic-AMP response element-binding protein and IKB kinase leading to NF-κB nuclear localization and the subsequent transcription of pro-survival genes such as Bcl-xL, caspase inhibitors and c-Myb [72,73]. Over-expression of Akt has anti-apoptotic effects in various cell types resulting in resistance to cell death [74].

4. CANCER CHEMOPREVENTION AND APOPTOSIS

Cancer is a pathologic condition where the normal mechanisms of cell cycle regulation are dysfunctional either due to excessive cell proliferation, insufficient apoptosis or both [75-77]. Suppression/inhibition of apoptosis during

carcinogenesis is known to play a role in the development and progression of cancers [77-79]. At present, it is accepted that cell populations are tightly regulated by their rates of proliferation, differentiation and death. When the homeostatic balance is disrupted in such a way that clonal outgrowth of mutated cell populations occurs, the development of a tumor will followed [77-79].

In simple terms, one can define carcinogenesis as a multistage process where a normal cell becomes transformed into one with a malignant phenotype. Cells become initiated by the acquisition of an activating mutation in an oncogene or an inactivating mutation in a tumor suppressor gene (initiation). Several additional factors confer these cells a growth advantage (promotion), which allow the cell to survive while accumulating abnormal characteristics and ultimately progressing to a metastatic tumor (progression) [80]. Carcinogenesis is a complex process driven by tight interactions between oncogene activation, tumor suppressor inactivation and cell death machinery. Early in transformation, activated oncogenes that drive the cell to uncontrolled proliferation simultaneously trigger apoptosis, probably as a safety mechanism to remove cells carrying oncogenic mutations [81]. Later in tumorigenesis, the supply of nutrients and oxygen becomes limited, with the tumor cells undergoing hypoxia-induced apoptosis [82]. In order to survive, tumor cells acquire apoptotic-inhibiting mutations (reduced apoptosis) [82]. Failures in normal apoptotic pathways contribute to carcinogenesis by creating a permissive environment for genetic instability and accumulation of mutations, promoting resistance to immune-based destruction, overriding cell-cycle checkpoints (that would normally induce apoptosis), facilitating growth factor/hormone-independent cell survival, supporting anchorage-independent survival during metastasis, reducing dependence of oxygen and nutrients, and conferring resistance to cytotoxic anticancer drugs and radiation [77]. Thus, inhibition of apoptosis can lead to tumor development.

In animal models, most chemical initiators are unable to initiate tumor growth unless a tumor promoter is subsequently applied. Many tumor promoters inhibit apoptosis *in vitro* [83]. Activation of apoptosis is thus being considered to be one of the most promising therapeutic approaches in cancer therapy [63,77,84]. Tumor cells can acquire resistance to apoptosis, for instance, by overexpressing anti-apoptotic proteins such as Bcl-2 or down-regulatinf/mutating pro-apoptotic proteins such as Bax, the expression of both being regulated by the p53 tumor suppressor gene [85,86]. p53 is a transcription factor essential for the prevention of cancer formation, which can be damaged by radiation, several chemicals and viruses such as human papillomavirus (HPV). The p53 pathway is ubiquitously lost in human cancer either by p53 gene mutation or by loss of cell signaling upstream and downstream of p53 in cancers that express the WT p53 gene [87]. Therefore, despite the enthusiasm towards apoptosis based-drugs, possible difficulties are also being anticipated such as selection of apoptosis-resistant tumor cells and systemic toxicity [84].

Several epidemiological studies, later evaluated by meta-analysis, have identified associations between certain dietary factors and cancer that either increase or decrease cancer risk [15,88,89]. It is currently accepted that diet can affect the overall process of carcinogenesis by different mechanisms: its constituents may contain cancer-causing substances as well as many cancer preventive agents. These dietary agents can retard or prevent the process of carcinogenesis by multiple mechanisms, namely 1) enhanced detoxification of the carcinogenic intermediates through induction of phase 2 drug metabolizing enzymes, 2) reduced carcinogenic activation due to suppression of cytochrome P450-dependent monooxygenases, 3) perturbations in cell cycle progression, 4) selective promotion of apoptosis in cancerous or precancerous cells, and 5) inhibition of angiogenesis and metastasis formation [16]. Since apoptosis provides a physiologic mechanism for eliminating abnormal cells, dietary factors affecting apoptosis can have an important effect on carcinogenesis. Conceivably, dietary factors that activate apoptosis in pre-cancerous cells offer a cancer preventive mechanism. In fact, most initiated cells are destroyed by apoptosis before they become malignant and develop into a tumor [79]. Increased understanding in the field of cancer has led to the conviction that most human malignancies should be fought on multiple fronts: in addition to cancer therapy, cancer prevention has become an important means of controlling cancer [8]. Common prevention strategies include avoiding exposure to known cancer-causing agents, enhancement of host-defense mechanisms against cancer, life style modifications and chemoprevention [8].

The term chemoprevention refers to the use of agents to slow the progression of, reverse or inhibit carcinogenesis, and was first introduced by Sporn and co-workers in the mid-1970's [90]. Animal studies, clinical trials and *in vitro* studies have examined the anticancer activity of numerous putative chemopreventive agents. These studies strongly suggest that the anti-cancer activities of many of these compounds involve the induction of apoptosis, and support the notion that apoptosis is a novel target for cancer chemoprevention [8,10]. Moreover, the pro-apoptotic properties of a variety of chemopreventive agents, like those of many conventional and experimental cancer chemotherapeutic agents, appear to be related to mitochondrial alterations in tumor cells [10,91]. In fact, several classes of chemopreventive agents contain members that trigger mitochondrial disruption and/or mitochondrial-mediated apoptosis (intrinsic pathway) in tumor cells *in*

