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Chemoprevention of Skin Cancer by Natural Compounds

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1. Introduction

Skin tumors in humans represent about 30% of all new cancers reported annually (Yusuf et al., 2007); their incidence is expected to increase substantially because of increased recreational exposure to sunlight and depletion of the ozone layer. The main factor incriminated in skin cancer is represented by the ultraviolet radiation, especially type B (UVB), which accounts for 90% of the skin cancer cases (Azis, 2005). Currently, between 2 and 3 million non-melanoma skin cancers and 132,000 melanoma skin cancers occur each year and one in every three cancers diagnosed is a skin cancer according to World Health Organization (WHO, 2004). Further more so, it is estimated that one in five persons will develop a basal cell or squamous cell carcinoma in its life time (Robinson, 2005). The identification of the critical molecular targets and signaling pathways involved in the development of premalign and malign lesions UV-induced and the development of agents that modulate these targets are important steps in the management of skin cancer. In 1894 Paul Gerson Unna established that there was a direct causal relationship between exposure to sunlight and the development of the skin carcinomas (Unna, 1894). The acute exposure to UV radiation leads to erythema, edema, burns, pain, thickening and pigmentation of the skin (Afaq & Mukhtar, 2001) while chronic exposure induces immunosuppression (Aziz, 2005), premature aging (Jurkiewicz et al, 1995; Lopez-Torres et al, 1998) and skin cancers (Katiyar & Mukhtar, 2001; Katiyar, 2008; Taylor et al, 1990). The sunlight UV spectrum can be separated into three wavelengths: UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm). Primarily UVA and UVB reach the earth’s surface as UVC is filtered out by the ozone layer (Shae & Parrish, 1991). Though the UVB radiations is a small part of UV emissions (4 - 5%), they are extremely aggressive being 1000 times more carcinogenic than UVA (Bowden, 2004; Mukhtar & Elmets, 1996). UVB directly or through the reactive oxygen species (ROS) affects the genetic material of exposed cells forming photoproducts such as DNA cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone (Afaq et al, 2007) respective 8-oxo-7, 8-dihydroguanine (8-oxo-dG) and 8-hydroxy-20-deoxyguanosine (8-OHdG) (Kawachi et al, 2008). Multistage carcinogenesis is a widely accepted hypothesis for the development of skin cancer. Skin carcinogenesis is divided into three stages namely: initiation, promotion and progression. Both UVA and UVB radiation have been shown to be complete carcinogens, capable of initiation, promotion and progression of carcinogenetic process (Aziz et al, 2005; Brash et al, 1996; Elmets et al, 2001). Actually, UV has dual actions in the development of skin cancers, it damages DNA and induces mutations of cellular genes crucial for oncogenesis; it induces immunosuppression, which prevents tumor rejection by the host. To exert its biological effects, UV must be first absorbed by cellular...
chromophore, which transforms the energy into a biochemical signal. Nucleic acids and proteins, especially tryptophan and tyrosine, are the major cellular chromophores in skin. In the DNA molecule, after the absorption of UVB radiation, dimeric photoproducts between adjacent pyrimidine bases are formed (Afaq et al, 2007). Usually the photoproducts formed are repaired effectively, but if the solar exposure is chronic and excessive, the process of repair is exceeded, the photoproducts persist and replicate, which may lead to transcriptional errors and, finally, to cancer (Kane & Kumar, 1999). The most frequent DNA mutations irradiation include the substitution of cytosine base by thymine (Mitchell et al, 1999). It was noticed that the methylation of the cytosine residues in the 5'-CCG and 5'-TCG sequences increased the formation of CPDs over 10 times, preferentially in p53 gene (Lou et al, 2001). Disregulated sunburn apoptosis together with loss of normal function of the product of the p53 gene may lead to transcriptional errors and, finally, to cancer. In addition, UVB radiation produces reactive oxygen species that can also damage DNA molecules and generate mainly 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Ahmed et al, 1999). Formation of 8-OHdG induces G-C→T-A transversions during DNA replication especially in ras and p53 genes initiating the carcinogenic process (Basset-Seguin et al, 1994). Changes in the gene expression after UV irradiation are grouped as follows: initial changes (from 0.5 to 2 h), intermediate changes (from 4 to 8 h), and late ones (from 16 to 24 h). Firstly, UV activates several transcription factors - junB, junD, c-fos, ETR101, EGR1, and URY, up-regulates several mitochondrial proteins involved in the removal of reactive oxygen species - and suppresses c-Myc (Mlakar & Glava, 2007). The intermediate phase shows expression of genes of chemokines, cytokines, and growth factors (IL-8, Gro-α, Gro-β, MDNCF, and MIP2-β). In the late phase, the strongest induced genes are: keratinocyte differential markers, p53 and p21WAF1, ERK3, growth arrest and DNA-damage-induced protein (GADD45) (Murakami et al, 2001). The DNA damage response culminates in activation of cell cycle checkpoints and the appropriate DNA repair pathways or, in certain contexts, initiation of apoptotic programs. Progression of normal cell division depends on cyclin interaction with cyclin dependent kinase (CDKs) and the degradation of cyclins before chromosomal segregation. Derepression of CDK-cyclin complexes or CDKs regulators result in continued proliferation or unscheduled re-entry into the cell cycle. There is ample evidence that UVB induces reactive oxygen species which have been involved in the pathogenesis of skin cancer (Bartsch & Nair, 2004; Halliday, 2005; Trouba et al, 2002). UVB exposure induces generation of reactive oxygen species in keratinocytes and fibroblasts: superoxide anion radical (O₂⁻), hydrogen peroxide (H₂O₂), the hydroxyl radical (HO) and singlet oxygen (1O₂). An imbalance between the generation of ROS and cellular antioxidant capacity leads to a state of oxidative stress that contributes to various pathological conditions including cancer (Katiyar & Elmets, 2001). Beside ROS, reactive nitrogen species (RNS), including nitric oxide (NO⁻), play a role in oxidative damage of proteins via nitrosylation reactions. More importantly, NO can react with superoxide and produce peroxynitrite anion (ONOO⁻), a toxic product involved in the apoptosis process and DNA cleavage (Katiyar et al, 2001). Defence mechanisms to remove the ROS include enzymes such as: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), heme oxygenase-1 (HO-1), metallothionein-2 (MT-2), glutathione S-transferases (GSTs), and small molecules with antioxidant activity such as: glutathione reduced (GSH), vitamin C, E, ubiquinona, β-carotene, etc., (Droge, 2002). Superoxide dismutase belongs to major antioxidant enzymes that contribute to the homeostasis of oxygen radicals in the skin. The dismutation of superoxide by SOD results in the production of hydrogen peroxide, which is subsequently converted to water and oxygen through a reaction catalyzed by CAT. An imbalance in the ratio of antioxidant enzymes may thus contribute to an excessive accumulation of ROS.
increasing oxidative stress and skin damage (Sharma et al., 2004). GPx is a selenoprotein that catalyses the conversion of UV-induced hydrogen peroxide into water and molecular oxygen using GSH as unique hydrogen donor. Besides the regulatory effect of GSH on the activity of redox sensitive cysteine-containing enzymes (Klatt & Lamas, 2000), it modulates the activation and binding of transcription factors. The cellular concentration of the GSH is up to 100 fold higher than glutathione oxidized (GSSG) so, minor increase in GSH oxidation can significantly affect the GSH:GSSG ratio and consequently influences signal transduction and cell cycle progression (Klaunig & Kamendulis, 2004). The balance can be restored by NADPH-dependent glutathione reductase or the thioredoxin/glutaredoxin systems that catalyze the reduction of GSSG to GSH or by elimination of the GSSG from the cell (Schafer & Buettner, 2001). In addition, GSH contributes to the regeneration of ascorbate and α-tocopherol and detoxifies reactive species via its ability to conjugate pro-oxidants (Heck et al., 2003). Nrf2 (Nuclear factor-erythroid 2-related factor) and Keap1 (Kelch-like-ECH-associated protein 1) are key proteins in the coordinated transcriptional induction of various antioxidant-metabolizing enzymes such as: glutamate cysteine synthetase cysteine/glutamate exchange transporter (Wild et al., 1999), glutathione S-transferase, nicotinamide adenine dinucleotide phosphate quinone oxidoreductase-1 (NQO1), HO-1 (Alam et al., 1999) and thioredoxin (Ishii et al., 1999). Catalase, SOD and GPx are also dependent on Nrf2 (Kawachi et al., 2008). Overexpression of Nrf2 was shown to protect the cells from Fas-induced apoptosis (Kotlo et al., 2003) because it reduce the export of glutathione from apoptotic cells and increase intracellular GSH levels (Morito et al., 2003). In addition, the Nrf2–Keap1 pathway inhibit activation of activator protein 1 (AP-1) and nuclear factor kappa B (NF-kB) pathways, transcription factors involved in the inflammatory processes (Ichihashi, 2003; Li & Nel, 2006). A recent study indicated that p53, which is a key molecule in UV-induced apoptosis, suppressed the Nrf2-dependent transcription of antioxidant response genes (ARE) (Faraonio et al., 2006). Several cytosolic kinases such as protein kinase C (PKC)(Huang et al., 2002), mitogen-activated protein kinase (MAPK), p38 (Alam et al., 2000), and phosphatidylinositol-3-kinase (PI3K) (Kang et al., 2002) have been shown to modify Nrf2 and participate in the mechanism of signal transduction from antioxidants to the ARE. ROS also affect regulation of the gene expression of signaling molecules such as MAPKs and interleukin inflammatory cytokines as NF-kB and AP-1 (Katiyar & Mukhtar, 2001). MAPKs are components of kinase cascades that connect extracellular stimuli to specific transcription factors, thereby converting these signals into cellular responses. In mammalian systems, there are three subgroups of MAPKs: extracellular-signal-regulated kinases (ERKs), Jun N-terminal Kinases (JNKs), and p38 mitogen activated protein kinase (Chang & Karin, 2001). Once activated, these serine/threonine kinases translocate to the nucleus and phosphorylate AP-1 and NF-kB (Bode & Dong, 2003). AP-1 regulates the expression of some cellular cycle regulatory proteins, such as cycline D1, p53, p21, p19 (Aggarwal & Shishodia, 2006). The activation of NF-kB increases the expression of some pro-inflammatory cytokines, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Baldwin, 2001). Several reports published within the last decade showed that activation of NF-kB promoted cell survival and proliferation and down-regulation of NF-kB sensitized the cells to apoptosis induction (Durai & Aggarwal, 2004). The genes NF-kB up-regulated including antiapoptotic proteins (Bcl-2, Bcl-XL), inhibitor of apoptosis (cIAP), survivin, TNF receptor-associated factors (TRAF1, TRAF2) have been reported to block apoptosis pathway (Garg & Aggarwal, 2002). ROS generated by UV radiation also induce matrix metalloproteinases (MMPs) and inactivate their tissue inhibitors, resulting in increased levels of matrix degrading enzymes, a key feature of photoaged skin. A cellular mechanism for the elimination of UV-damaged skin cells is to initiate apoptosis. Apoptosis is controlled by
two signaling pathways, the extrinsic or death receptor-mediated pathway and the intrinsic or mitochondrial-mediated pathway. The death receptor-mediated pathway is initiated by the interaction of the ligand with its receptor, which leads to the activation of caspase 8 and 3. In contrast, the intrinsic pathway of apoptosis relies primarily on the permeabilization of mitochondrial membranes, with associated release of cytochrome c, leading to activation of caspase 9 and cleavage of caspase 3, 6, or 7. These downstream caspases induce in turn the cleavage of protein kinases and cytoskeletal proteins affecting cytoskeletal structure, the cell cycle regulatory effect and the related signaling pathways. DNA fragmentation, chromatin condensation, membrane blebbing cell shrinkage finally occurs. Recent studies indicate a third option to induce apoptosis namely one mediated by the endoplasmic reticulum. Strong stressing agents such as: UV radiation, chemotherapy, and peroxinitrite, determine the overexpression of DNA damage-inducible gene 153 (GADD153) and induce dephosphorylation of the pro-apoptotic protein Bad and down-regulation of Bcl-2 expression, enabling apoptosis (Tagawa et al, 2008). The extrinsic and intrinsic apoptotic pathways are regulated by several proteins such as NF-κB, Bcl-2 family proteins and the inhibitors of apoptosis (IAPs). An important component of IAP is survivin, the overexpressed protein in embryonic tissues and in tumors (Altieri, 2003). Because its level correlates with tumor angiogenesis, chemoresistance, tumor relapse and poor prognosis is considered as a potential molecular target for cancer therapy. The tumor suppressor protein p53 is a transcription factor that plays an essential role in the cellular response to UV. By blocking the cell cycle in cells which have suffered an excessive DNA damage, p53 prevents replication of damaged DNA as long as it has not been repaired. In case of unsuccessful reparation, p53 induces apoptosis via induction of p21waf1/cip1, Bax, apoptotic protease activating factor-1 (Apaf-1) and the caspase cascade (Amin et al, 2009).

In the process of carcinogenesis, the immunosuppressive effect of the UV radiation has to be taken into account. UV-induced inflammation is considered an early event in tumor promotion and/or tumor development (Katiyar, 2008). Similar to many chemical tumor promoters, UV also elicits NF-κB-mediated inflammation and changes in the expression of genes associated with cell proliferation and differentiation, as well as prostaglandin and cytokine production. Pro-inflammatory cytokines such as interleukin IL-1β, IL-6, IL-10 and tumor necrosis factor-α (TNF-α) impair the antigen presenting abilities of Langerhans cells which may result in suppression of the immune response (Susan et al, 2007). The inflammatory cells produce ROS and IL-10 which increase DNA oxidative damage and induce tumor promotion (Mukhtar H & Elmets, 1996). In addition, the UV radiation decreases the number of circulating T lymphocytes and the T helper/T suppressors’ ratio and it alters the immune function of tumor cells (Patrick & Noah, 2007). Several studies showed using various experimental models of tumorigenesis that TNF-α and IL are involved in tumor promotion (Surh, et al, 2005). As a response to inflammatory stimuli and under the influence of COX, prostaglandins (PG) are produced in the skin, especially PGE2 and PGF2, molecules that play a role in tumor promotion (Narisawa et la, 1997). Another inflammation mediator is NO. NO released by iNOS is involved in tumor promotion; this fact is supported by the suppressive effect of aminoguanidine, an iNOS inhibitor on tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced mouse skin tumor formation (Chun et al, 2004). It was showed that CPDs induced immunosuppression through cytokines production such as IL-10 or through the inhibition of transcription factors (Amerio et al, 2009). Photoisomerization of urocanic acid, which is found in high concentrations in the stratum corneum, may play a role in skin immunosupresion. For this reason, the prevention of UV-induced immunosuppresion represents an important strategy in the
management of skin cancers. Considering that UV induces oxidative stress-mediated adverse effects in the skin, the regular intake or topical application of antioxidants is suggested to be a useful way to reduce the harmful effects of UV radiation. These agents could prevent the formation of initiated cells or eliminate the initiated cells reducing the risk of cancer development.

2. Chemoprevention in skin cancers

The continue increase in the incidence of skin neoplasia, especially the non-melanoma ones, justifies the permanent preoccupation of the medical world to find an optimal solution for prevention. Chemoprevention, defined as “the use of agents capable of ameliorating the adverse effects of UVB on the skin” by natural compounds, represents a new concept in the attempt to control the carcinogenesis process (Zhao et al, 1999). This concept may be understood as a way to control tumor development by which the tumor evolution is slowed down or stopped; this control is realized by natural products or synthetic chemical compounds. The beginning of chemoprevention date back to 1920 but it was relaunched in 1970 by the research performed by Sporn (Sporn et al, 1976). The fruits, vegetables and plants with different pharmacological properties present rich sources of chemical substances with a chemopreventive role (Zhao et al, 1999). In fact, the role of chemoprevention is to interrupt the cascade of aberrant cellular signals and to stop the carcinogenic process. It seems that more than one dietary product has a chemopreventive role by modulating one or more signaling pathways in the cellular proliferation process. Exploring of the action mechanism of these compounds through in vivo studies to identify the target molecules and signaling pathways is critical in order to evaluate their clinical applicability. Taking into account the epidemiological data and the studies on animal models we can state that there are 30 new categories of clinical substances included in clinical trials as photochemopreventive agents. Some of them are antioxidants (vitamin E, ascorbic acid, polyphenols, isoflavonoids), which protect the skin from the oxidative effects; others interfere with the process of DNA repair or modulate the immunosuppression induced by UV radiation.

2.1 Signaling pathways and target molecules involved in chemoprevention

The cell signaling pathways activated by natural dietary agents are numerous and different for different agents. The inhibitory effects of natural compounds in skin carcinogenesis have been attributed mainly to the biologic activities of the polyphenolic fractions. Polyphenols have antioxidant effects; they reduce the DNA oxidative damage, formation of lipid peroxides and carbonyl proteins and inhibit UVB-induced oxidative stress-mediated phosphorylation of MAPK signaling pathways. Polyphenols are known to inhibit the protein tyrosine kinase activities of epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), inhibit UVB-induced phosphatidylinositol-3-kinase (PI3K) activation, blocked cell cycle progression at G1 phase and inhibited the activities of CDK-2 and CDK-4 in a dose-dependent manner. Also, blocked the activation of NF-kB and AP-1 and inhibited expression of COX-2 and iNOS. All these qualities make polyphenols an important option in chemoprevention. In the following sections we will focus on the mechanisms involved in the antiproliferative activity of natural products in skin cancer. The figure 1 presents the molecular targets of natural compounds in chemoprevention.
Fig. 1. Molecular targets of natural compounds in chemoprevention. The effects of natural compounds in skin carcinogenesis are variable: reduce inflammation, angiogenesis and oxidative stress, inhibit DNA damage, immunosuppression and modulate the signaling pathways involved in the development of skin cancers. Abbreviations: MAPK - mitogen activated protein kinase, ERK1/2 - extracellular-signal-regulated kinases, JNK - Jun N-terminal Kinase, p38 - p38 mitogen activated protein kinases, AP-1 - activator protein 1, NF-kB - nuclear factor kappa B, COX-2 - cyclooxygenase 2, AKT - serine/threonine protein kinase, PI3K - phosphatidylinositol-3-kinase, MMP-3 - metalloproteases, IL - interleukin, RTKs - receptor tyrosine kinases, EGFR - epidermal growth factor receptor, PDGFR - platelet-derived growth factor receptor, FGFR - fibroblast growth factor receptor, TGFR - transforming growth factor receptor, IGFR - insulin-like growth factor receptor, SOD - superoxide dismutase, CAT - catalase, GPx - glutathione peroxidase, IKK - IκB kinase, CDK - cyclin-dependent kinases, LO - lipooxigenase, iNOS - inducible nitric oxide synthase, PCNA - proliferating cell nuclear antigen, GADD45 - growth arrest and DNA damage, p53- tumor suppressor protein, p21, p27 - cyclin-dependent kinase inhibitors.

2.1.1 Receptor tyrosine kinases signaling
Receptor tyrosine kinases (RTKs) are important regulators involved in controlling cell proliferation, differentiation, survival, migration and metabolism (Schlessinger & Lemmon, 2006). The RTKs families include EGFR, PDGFR, FGFR, transforming growth factor receptors (TGF), insulin-like growth factor receptors (IGFR). About 15 years back, curcumin, a natural agent, was reported to inhibit EGFR intrinsic kinase activity in human epidermoid carcinoma A431 cells (Korutla & Kumar, 1994). In in vitro assay, EGCG also strongly inhibited the protein tyrosine kinase activities of EGFR, PDGFR and FGFR (Lin, 2002). On another in vitro experimental model, EGCG could reduce the autophosphorylation level of EGFR and blocked of EGF binding to its receptor. Nomura et al. demonstrated that EGCG inhibited also the activation of PI3K and AKT phosphorylation UVB-induced in epidermis of mice (Nomura et
Based on these findings it could be concluded that modulation of RTKs signaling might be involved in antiproliferative effects of natural compounds.

2.1.2 Signal transducers and activators of transcription pathway
Signal transducers and activators of transcription (STAT) proteins function as downstream effectors of signaling, which control cell proliferation, survival, apoptosis and differentiation (Kundu et al, 2009). STAT-1, STAT-3 and STAT-5 can be considered as molecular markers for early detection of certain types of tumors, as well as prognostic factors and predictors of response to various types of therapy (Buetnner et al, 2008). Several dietary agents, such as green tea, resveratrol and curcumin have been shown to suppress STAT activation in tumor cells. So, green tea reduced the binding of STAT-1 to ADN by reducing the phosphorylation of protein I without involving its antioxidant role (Tedeschi et al, 2004). EGCG has also been shown to down-regulate the phosphorylation of STAT-3 (Masuda et al, 2001) while curcumin inhibited IL-6-induced STAT-3 and IFN-α-induced STAT-1 phosphorylation (Schnyder et al, 2002). These data provide further arguments in favor of the therapeutic effect of natural agents which suppress STAT activation.

2.1.3 Cell-cycle regulatory signaling
Several dietary agents including curcumin, resveratrol, genistein, dietary isothiocyanates, apigenin, and silybin have been shown to interfere with the abnormal progression of cell cycle regulation in cancer cells (Lapenna & Giordano, 2009; Schwartz & Shah, 2005). Curcumin has been shown to inhibit progression of the cell cycle by down-regulating the expression of cyclin D1 at the transcriptional and posttranscriptional level, most probably by suppressing the activity of NF-kB (Aggarwal & Shishodia, 2006). As a consequence, it inhibited cell proliferation and induced apoptosis. Resveratrol inhibited UV-mediated increase in proliferating cell nuclear antigen (PCNA), CDK 2, 4 and 6, cyclins (D1 and D2), and the MAPks in SKH-1 hairless mice. On cell lines of epidermoid carcinoma (A431) Ahmad et al. showed that resveratrol induced the blocking of the cellular cycle in the G1 phase and apoptosis via modulations in CDKIs-cyclin-CDK machinery (Ahmad et al, 2001). The authors showed that a retinoblastoma (Rb)-E2F/DP pathway was part of this mechanism. On SK-Mel-28 human melanoma cells, Larrosa et al. showed that resveratrol induced apoptosis, S phase arrest and up-regulation of cyclins A, E, and B1 (Larrosa et al, 2003). Another compound, carnosol, a phenolic diterpenes, present in rosemary, induced G2/M cell cycle arrest through altering the levels of cyclin A and cyclin B1 (Pan & Ho, 2008). Honokiol, a biphenolic compound and constituent of the bark and leaves of Magnolia plant species, caused cell cycle arrest in tumor cells by stimulating Kip1/p27 and Cip1/p21 and it reduced the protein levels of CDK 2, CDK 4 and CDK 6, cyclin D1, D2 and E (Vaid et al, 2010). These studies indicate that natural products can exert their antiproliferative effect by modulating CDKIs-cyclin-CDK complex.

2.1.4 Apoptotic regulators
Many natural chemopreventive agents induce cell cycle arrest or apoptosis by activating p53 and its target genes. Ahmad et al. performed the first studies regarding the role of EGCG in the apoptosis of tumor cells in 1997 (Ahmad et al, 1997). They observed that EGCG protected the normal cells by reducing the number of keratinocytes that were sunburned. In precancer lesions (papillomas) and invasive squamous carcinoma EGCG stimulated apoptosis (Chung et al, 2003). Several studies showed that green tea administered orally in hairless SKH-1 mice increased the number of p53 and p21 positive cells in epidermis after irradiation (Khan et al, 2006; Yusuf et al, 2007). Also, it was noticed that polyphenols
induced nuclear condensation, the activation of caspase 3 and the cleavage of poly (ADP-ribose) polymerase. They determined the oligomerization of Bax and the depolarization of the mitochondrial membrane, realizing the cytochrome c into the cytosol. These findings were supported by the fact that adding catalase to the cellular system prevented the apoptosis generated by EGCG (Khan et al, 2006). Babli et al. have shown that treatment with different concentrations of theaflavins and thearubigins from black tea on A375 cells reduced of Bcl-2 protein expression, and increased the Bax expression (Babli et al, 2008). The increased ratio of Bax/Bcl-2 proteins may be responsible for the induction of apoptosis in these cells.

Huang et al reported that resveratrol induced apoptosis in cells expressing wild-type p53, but not in p53-deficient cells (Huang et al, 1999). Resveratrol also, activated the expression of p21, p27, BAX, PUMA, MDM2, and cyclin G, all important downstream targets (Shankar et al, 2007). She et al. found that, in the JB6 mouse epidermal cell line, resveratrol activated ERK1/2, JNK, and p38 MAPK and induced serine-15 phosphorylation of p53 (She et al, 2001). In addition, resveratrol modulated the function of survivin, a biomarker of tumor promotion and a negative regulator of apoptosis (Bhardwaj et al, 2007). Several studies have shown that topical treatment with silymarin to SKH-1 hairless mice inhibited solar UV radiation-induced skin tumorigenesis. The compound inhibited the proliferation of epidermis cells by inducing the expression of p53 and p21/cip 1 in cells (Dhanalakshmi et al, 2004). It was shown that curcumin, another natural compound, induced apoptosis in human basal cell carcinoma by increasing the expressions of p53, p21Waf1 and GADD153 proteins. It seems that the most important effect of curcumin is the anticarcinogenic one mainly due to the reduction of cellular proliferation and induction of apoptosis in the tumor cells and less due to its antioxidant and antiinflammatory effect (Pan & Ho, 2008). Naringenin, a flavanone present in orange peel, is believed to contribute to the activation of phase II detoxifying enzymes by protecting against UVB-induced apoptosis in keratinocyte HaKAT cells. In addition, naringenin induces apoptosis in various cancer cells (El-Mahdy et al, 2008).

Most of the natural chemopreventive agents including curcumin, resveratrol, EGCG, lycopene, genistein, and luteolin act as inhibitors of NF-kB pathways (Aggarwal & Shishodia, 2006; Amin et al, 2009; Singh & Aggarwal, 1995). These compounds may block one or more steps in the NF-kB signaling pathway such as: inhibition of the most upstream growth factor receptors that activate the NF-kB signaling cascade, translocation of NF-kB to the nucleus, DNA binding of the dimers, or interactions with the transcription. The most known NF-kB target genes influenced by the natural chemopreventive agents include inhibition of Bcl-2, Bcl-xXL, cyclin D1, MMPs and VEGF (Shishodia et al, 2005). It seems that curcumin and curcuminoids mediate their therapeutic effects by modulating NF-kB target genes. Resveratrol inhibited TPA stimulated activation of AP-1 in mouse skin in vivo and, applied topically, attenuated NF-kB activation by blocking IKKβ activity (Cooper & Bowden, 2007). Curcumin also, suppressed the TNF-α dependent activation of IKK and as a result it inhibited the translocation of the p65 subunit. Also, it inhibited the activation of NF-kB mediated by phorbol esters and hydrogen peroxide (Singh & Aggarwal, 1995). The same inhibitory effects were induced by resveratrol on epithelial cells (Masuda et al, 2001) or on models of chemical induced carcinogenesis (Manna et al, 2000). Another compound, the caffeic acid, decreased the binding of the p50-p65 complex to ADN while other antioxidants such as sanguinarine and emodin blocked the degradation of IkBa as a response to TNF-α, phorbol esters and IL-1 (Chaturvedi et al, 1997). Yang sc. showed that green tea polyphenols and EGCG inhibited the activation of NF-kB by inhibiting the activity of IKK, a mechanism that explains their antiproliferative effect (Yang et al, 2002).
Ahmad et al. recently reported that EGCG treatment inhibited cell growth and induced apoptosis in human epidermoid carcinoma (A431) cells but not in normal human epidermal keratinocytes (NHEK) (Ahmad et al, 2000). The effect was due to a stronger inhibition of EGCG on tumor cells of TNF-α-mediated NF-κB activation compared to normal cells. It was shown that green tea polyphenols, quercetin, resveratrol, curcumin and capsaicin inhibited the activation of AP-1 by inhibiting the binding of AP-1 to DNA and as a consequence the transcriptional activation was inhibited. The effects of natural agents differ on the type of cells used. So, on human transformed bronchia cells EGCG inhibited c-Jun and ERK1/2 phosphorylation (Yang et al, 2000) while on normal keratinocytes EGCG increased the response to AP-1, a MAPKinasemediated effect (Balasubramanian et al, 2002). On HaKaT keratinocytes it was observed that EGCG blocked the activation of c-Fos induced by UVB and inhibited the activation of p38 (Chen et al, 1999). Recently, EGCG has been shown to inhibit 12-O-tetradecanoylphorbol-13-acetate or EGF-induced transformation of mouse epidermal cell line JB6 by the inhibition of AP-1 (Lu et al, 1994). In vitro it was shown that resveratrol inhibited the TNF-α dependent activation of AP-1 and as a consequence the activation of JNK and MEK kinases (Manna et al, 2000). Curcumin suppressed the expression of c-Jun and c-Fos in CD-1 mouse skin after treatment with TPA and the expression of COX-2 and PGE2 in the same model (Lu et al, 1994). Quercetin, another potent antioxidant, blocked the transcription of TNF-α induced by LPS in macrophages by inhibiting MAPKinasen and the binding of AP-1 to DNA (Wadsworth et al, 2001).

The dietary phytochemicals such as curcumin (Cho et al, 2005), resveratrol (Shih et al, 2002), and green tea polyphenols (Katiyar et al, 2001) have been shown to modulate the MAPKinasen. The first observations were made in vitro on epidermis microsomes from irradiated mice pre-treated with polyphenols. EGCG strongly inhibited the tyrosine kinase and MAPK activities in transformed mouse embryonic fibroblasts without effect in the normal cells (Wang & Bachrach, 2002). Pretreatment of normal human epidermal keratinocytes with EGCG reduced UVB-induced hydrogen peroxide production and its mediated phosphorylation of MAPK signaling pathways (Katiyar et al., 2001). Thus, EGCG inhibited oxidative stress-mediated phosphorylation of MAPK signaling pathways. During acute UVB exposure, silibinin inhibited the activation of MAPK and AKT, induced p53 and Cip1/p21 expression and suppressed DNA damage in mouse skin (Gu et al, 2005). Resveratrol also, modulated all three MAPKinasen, a variable effect depend on the cell type and the dose of resveratrol used. As a result, in some cells it activated MAPKinasen and inhibited it in others. Woo et al. showed that resveratrol inhibited MMP-9 expressions PMA-induced by inhibiting JNK (Woo et al, 2004). It seems, that the ability of curcumin to modulate the MAPK signaling pathway, might contribute to the inhibition of inflammation by curcumin. Ursolic acid also, inhibited the activity of lipooxygenases, the activation of STAT-3, the activation of c-Src, Janus 1, Janus 2 kinases and of the kinases regulated by extra-cellular signal (ERKs) (Hollosy et al, 2000). Another terpene carnosol, found in rosemary and sage leaves, inhibited LPS-induced activation of p38 and p44/42 MAPK and IKK, and reduced LPS-induced iNOS expression (Lo et al, 2002). Moreover, carnosol reduced invasion in B16/F10 melanoma cells, possibly through inhibition of NF-kB and c-Jun, and by blocking MMP-2 and MMP-9 activity (Huang et al, 2005).

2.1.5 Antioxidative effects
Tea polyphenols are strong scavengers against superoxide, hydrogen peroxide, hydroxyl radicals, nitric oxide and peroxynitrite produced by various chemicals and biological systems.
It has been shown that polyphenols from green tea administered topically or in the drinking water reduced the proliferation of experimental tumors in a dose-dependent manner and induced partial regression of skin papillomas in SKH-1 mice (Katiyar et al, 2001). This effect was due to reduction the DNA damage. Polyphenols decreased the generation of CPDs in the skin and protected against UV-induced depletion of antioxidant-defense enzymes. Green tea polyphenols have been reported to induce transcription of ARE (antioxidant-responsive element) dependent reporter genes and also to strongly activate ERK2 and JNK1 (Bode & Dong, 2003). This activation was shown to be associated with increased mRNA levels of the immediate-early response genes, c-jun and c-fos. EGCG, the most potent compound of green tea, protected against UV-induced depletion of GSH level and GPx activity in human skin (Katiyar et al, 2001), restored detoxification enzymes SOD and CAT on models of chemical-induced carcinogenesis (Saha & Das, 2002). Further more, EGCG treatment inhibited UVB-induced depletion of GSH level and GPx activity in human skin (Katiyar et al, 2001), restored detoxification enzymes SOD and CAT on models of chemical-induced carcinogenesis (Saha & Das, 2002). Further more, EGCG treatment inhibited UVB-induced depletion of GSH level and GPx activity in human skin (Katiyar et al, 2001), restored detoxification enzymes SOD and CAT on models of chemical-induced carcinogenesis (Saha & Das, 2002).
depends on its concentration. Whereas low concentrations of curcumin (<20 μM) decreased ROS production, higher concentrations favored ROS generation (Chen et al, 2005).

2.1.6 Anti-inflammatory activity
It was affirmed that chronic inflammation though the recruitment of inflammatory cells, the release of cytokines and ROS created a micromedium favorable to cellular proliferation and carcinogenesis. On a model of contact hypersensitivity induced in mice by topical or systemic administration of 2,4-dinitrofluorobenzen it was observed that the proantocyanidins had a protective effect on the UVB-induced immunosuppression. Proanthocyanidins also inhibited carrageenan-induced paw edema in rats and suppressed LPS-induced inflammation (Ho et al, 2007). Quercetin has anti-inflammatory effect due to the inhibition of lipooxygenase, cyclooxygenase, and PKC, resulting in a reduction of the production of pro-inflammatory mediators (Pan & Ho, 2008). More recently, silibinin has been found to inhibit inflammation and angiogenesis in SKH-1 hairless mice and protect from photocarcinogenesis by modulation of cell cycle regulators, MAPK and AKT signaling pathways (Gu et al, 2005).

2.1.7 COX-2
Several dietary components including galangin, luteolin, apigenin, genistein, green tea catechins, curcumin, and resveratrol have been shown to suppress COX-2. The inhibition took place at a transcriptional level. Curcumin was one of the first chemopreventive phytochemicals shown to possess significant COX-2 inhibiting activity through the suppression of NF-kB (Kundu et al, 2009). A similar effect was observed in delphinidin, an anthocyanidin present in dark fruit (Afaq et al, 2007). It inhibited the COX-2 expression by blocking MAPK signaling and NF-kB, AP-1 and C/EBPd nuclear translocation and conferred protection against UVB-mediated oxidative stress and apoptosis in mouse skin. It was found that the treatment with silymarin and green tea extract inhibited UVB-induced sunburn cell formation, COX and ornithine decarboxylase activities and ornithine decarboxylase mRNA expression (Katiyar et al, 1997). Moreover, pretreatment with green tea extract inhibited COX-2 expression induced by TPA in mouse skin and assured a good protection against erythema, immunosuppression and photoaging of the irradiated skin. Also, EGCG treatment reduced UVB-induced production of PG metabolites, such as PGE2, PGF2a and PGD2, molecules involved in tumor promotion. Topical application of oligonol-G, an innovative formulation composed of catechin-type oligomeric polyphenols, lowered chemically induced tumor promotion, UVB-induced lipid peroxidation, and 12-O-tetradecanoylphorbol-13-acetate (TPA) or UVB-induced COX-2 expression in mouse skin (Kundu et al., 2008). The oral administration of oligonol-L or oligonol-G had the same effect as the topical treatment on COX-2 expression in UVB irradiated hairless mouse skin (Kundu et al, 2009). The effect was mediated by the inhibition of ERK1/2 and p38 MAPkinase activation. Pretreatment with caffeine inhibited phosphorylation of AKT and up-regulation of COX-2, two critical oncogenic pathways in skin tumorigenesis. Also, it increased UVB-induced apoptosis in HaKaT cells, and blocked UVB-induced CDK1 phosphorylation (Han et al, 2011).

2.1.8 MMPs
Recent studies have indicated that the MAPkinase signal transduction pathways play an important role in regulating a variety of cellular functions including MMP expression and cell growth (Cho et al, 2007). It was shown that MMP-9 expression was regulated by JNK- and ERK-dependent signaling pathways; the regulatory effect was inhibited by the administration
of de green tea polyphenols and black tea extract (Gum et al, 1997). EGCG has been also, reported to inhibit the MMP-2 and MMP-9 and prevent UVB-induced suppression of the immune system in mice. Administered orally, the green tea extract inhibited UV carcinogenesis, reduced the expression of MMP-2 and MMP-9 in parallel with an increase in the expression of the TIMP-1 inhibitor (Mantena et al, 2005). Moreover, it was observed that its effect was accompanied by a reduction in the expression of vascular endothelial cell antigens, such as CD31 and VEGF, in the UVB-induced tumors. Curcumin was shown to down-regulate the expression of MMP-2 and MMP-9 (Heng, 2010). It was known as MMP-2 and MMP-9 are responsible for digestion of collagen IV in basement membranes and subendothelial collagen V, allowing the tumor cells to invade the dermis, as well as penetrate blood vessels.

3. Botanicals antioxidants used in chemoprevention

Of all the chemopreventive agents polyphenols enjoy the most attention as they are found in a great variety of fruits and plants and have proven their complex effects in both in vivo and in vitro studies. In the table are presented the main phytochemicals used in chemopreventions of skin cancer, the experimental models studied and the mechanisms involved.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Active principals</th>
<th>Experimental model</th>
<th>Target/Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcuma longa</td>
<td>curcumin</td>
<td>DMBA-TPA-induced papillomas</td>
<td>↓ MAPK, ↓ NF-kb (Chun et al, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMBA-induced papillomas</td>
<td>antioxidant (Soudamini &amp; Kuttan, 1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD-1 mice/TPA</td>
<td>↓ COX (Ishizaki et al, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEC cell</td>
<td>↓ caspases, ↓ cytochrome c (Chan et al, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 431, HaKaT cell</td>
<td>↓ COX, AP-I, ↑ caspases, cytochrome c (Cho et al, 2005)</td>
</tr>
<tr>
<td>Ocimum sanctum</td>
<td>leaf extract</td>
<td>DMBA-induced skin tumors in mice</td>
<td>antioxidant (Prashar et al, 1994)</td>
</tr>
<tr>
<td>Ocimum gratissimum</td>
<td>clocicum oil</td>
<td>DMBA-induced skin papillomas</td>
<td>antioxidant, ↑ -SH, ↑ cytochrome b5 (Singh et al, 1999).</td>
</tr>
<tr>
<td>Zingibe officinale</td>
<td>gingerol</td>
<td>B6 cells</td>
<td>↓ AP-1 (Bode et al, 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HaKaT cell</td>
<td>↓ ROS, ↑ caspases, ↓ COX-2, ↓ NF-kb (Kim et al, 2007)</td>
</tr>
<tr>
<td>Silybum marianum</td>
<td>silibinin</td>
<td>SENCAR mouse</td>
<td>↓ tumors, ↓ iNOS, ↓ COX-2, ↓ NF-kb (Gu et al, 2005); ↓ CFDs, ↓ p53 (Dhanaalashmi 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SKH-1</td>
<td>↓ COX-2, ↓ NF-KB (Kim et al, 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3H/HeN</td>
<td>↓ IL-10, ↓ IL-12, ↓ ROS, ↓ CD 11b+ (Katiyar et al, 2008)</td>
</tr>
<tr>
<td>fruits</td>
<td>apigenin</td>
<td>SKH-1</td>
<td>↓ ODC, p53, cell cycle regulatory proteins</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Svobodova et al, 2003)</td>
</tr>
<tr>
<td>Rosemarinus officinalis and Salvia officinalis</td>
<td>carnosul, acid ursolic</td>
<td>mouse</td>
<td>↓ DMBA-initiation, TPA-promotion, ↓ mARN MMP-1 (Svobodova et al, 2003)</td>
</tr>
<tr>
<td>Ginko biloba</td>
<td>ginko biloba extract</td>
<td>mouse</td>
<td>↓ SOD, ↓ CAT in skin</td>
</tr>
</tbody>
</table>

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Table 1. Molecular mechanisms involved in chemopreventive activity of natural products.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Active principals</th>
<th>Experimental model</th>
<th>Target/Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanguinaria canadensis</td>
<td>sanguinarine</td>
<td>A 431</td>
<td>↑ apoptosis (Ahsan et al, 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SKH-1</td>
<td>↓ edema, ↓ hyperplasia, ↓ PCNA, ↓ ODC (Ahsan et al, 2007)</td>
</tr>
<tr>
<td>Soy isoflavones</td>
<td>genistein</td>
<td>hairless mice</td>
<td>↓ expression of protooncogene and skin tumorigenesis (Svobodova et al, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SENCAR</td>
<td>c-fos, c-jun (Wang et al, 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SKH-1</td>
<td>↓ H2O2, ↓ LPO, ↓ 8-OHdG (Wei et al, 2002)</td>
</tr>
<tr>
<td>Prunus persica</td>
<td>kaempferol</td>
<td>in vitro</td>
<td>↓ apoptosis, ↓ DNA damage, ↑ LPO (Svobodova et al, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>guinea pigs</td>
<td>↓ edema</td>
</tr>
<tr>
<td>Punica graminatum</td>
<td>delphinidin</td>
<td>HaKaT</td>
<td>↓ LPO, ↓ PCNA, ↓ 8-HOdG (Afaq et al, 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SKH-1</td>
<td>↓ tumor development</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>proanthocyanidins</td>
<td>DMBA-TPA-induced skin tumorigenesis</td>
<td>↓ ODC, ↓ myeloperoxidase, ↓ PKC (Bomser et al, 2000)</td>
</tr>
<tr>
<td></td>
<td>polyphenols</td>
<td>DMBA-TPA-induced skin carcinogenesis</td>
<td>antioxidant (Zhao, et al, 1999)</td>
</tr>
<tr>
<td></td>
<td>resveratrol</td>
<td>SKH-1</td>
<td>↓ survivin, ↑ Smac/DIABLO (Aziz, et al, 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ edema, ↓ H2O2, ↓ infiltration of leucocytes, ↓ COX, ↓ NF-kB (Afaq et al, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ ROS, ↓ MAPK, ↓ NF-kB (Katiyar 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NHEK</td>
<td>↓ NF-kB (Adhami et al, 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>in vitro antioxidant, antiinflammamotory, antiproliferative (Dong, 2003)</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>root extract</td>
<td>DMBA-induced papillomas in mice</td>
<td>antioxidant (Prakash et al, 2002)</td>
</tr>
<tr>
<td>Canellia sinensis</td>
<td>EGCG, polyphenols</td>
<td>Balb/c</td>
<td>↓ incidence, multiplicity and volume of tumors (Katiyar et al, 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3H/HeN mice</td>
<td>↑ IL-12 and prevent immunosuppression UV-induced (Ahmad et al, 2000)</td>
</tr>
</tbody>
</table>


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4. Clinical trials

Due to the growing literature on natural compounds efficacy against a variety of cancers, several human trials using these agents have been conducted in the recent years. Epidemiological studies support a protective role of flavonoids, lycopene, lutein and various nutrient combinations administered orally or topically against DNA-damage. However, the data are not entirely consistent. Linden et al. used topical epigallocatechin gallate in a randomized, double-blind, placebo-controlled phase II clinical trial in the prevention of nonmelanoma skin cancer (Linden et al, 2003). Other dietary compounds, including silibinin, genistein, curcumin and resveratrol, have been recognized as cancer chemopreventive agents but their role in humans is still limited.

5. Conclusions and perspectives

UV irradiation of skin causes a number of cellular and pathological changes, including DNA damage, cell-cycle arrest, formation of sunburn cells (apoptosis), depletion of the antioxidant defense system, release of proinflammatory cytokines and immunosuppressive effects. The combination of these events can lead to cancer development. Epidemiologic and animal studies have identified the associations between certain diets and modulation of cancer risk. Diet/nutrition can exacerbate or interfere with carcinogenesis through genetic and epigenetic modulation, leading to altered cellular phenotypes and differing cancer outcomes. Thus, dietary intervention appears to be a promising strategy to prevent carcinogenesis. Further more, the natural compounds are non toxic agents and their using in therapy may also decrease the systemic toxicity caused by chemotherapy or radiotherapy. Understanding the molecular mechanisms of their action and effects on cellular signaling processes, as well as their structure-activity relationships is necessary for the generation of new, more effective derivatives. Further investigation of optimal doses and mechanism of protection are needed to better target and prevent photodamage with dietary and/or topical treatments.

6. Acknowledgment

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7. References


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Chemoprevention of Skin Cancer by Natural Compounds

through Activation of Cytotoxic T Cells and Inhibition of Angiogenesis in Tumors. J Nutr, 135, 2871–287.


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Skin cancers are the fastest growing type of cancer in the United States and represent the most commonly diagnosed malignancy, surpassing lung, breast, colorectal and prostate cancer. In Europe, the British Isles have been the highest rates of skin cancer in children and adolescents. The overall idea of this book is to provide the reader with up to date information on the possible tools to use for prevention, diagnosis and treatment of skin cancer. Three main issues are discussed: risk factors, new diagnostic tools for prevention and strategies for prevention and treatment of skin cancer using natural compounds or nano-particle drug delivery and photodynamic therapy.

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