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Soy isoflavone phyto-pharmaceuticals in interleukin-6 affections Multi-purpose nutraceuticals at the crossroad of hormone replacement, anti-cancer and anti-inflammatory therapy

Nathalie Dijsselbloem, Wim Vanden Berghe, An De Naeyer, Guy Haegeman*

Laboratory for Eukaryotic Gene Expression and Signal Transduction (LEGEST), Department of Molecular Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

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Abstract

Interleukin-6 is a pleiotropic cytokine which plays a crucial role in immune physiology and is tightly controlled by hormonal feedback mechanisms. After menopause or andropause, loss of the normally inhibiting sex steroids (estrogen, testosterone) results in elevated IL6 levels that are further progressively increasing with age. Interestingly, excessive IL6 production promotes tumorigenesis (breast, prostate, lung, colon, ovarian), and accounts for several disease-associated pathologies and phenotypical changes of advanced age, such as osteoporosis, rheumatoid arthritis, multiple myeloma, neurodegenerative diseases and frailty. In this respect, pharmacological modulation of IL6 gene expression levels may have therapeutical benefit in preventing cancer progression, ageing discomforts and restoring immune homeostasis. Although ''plant extracts'' are used in folk medicine within living memory, it is only since the 20th century that numerous scientific investigations have been performed to discover potential health-protective food compounds or ''nutraceuticals'' which might prevent cancer and ageing diseases. About 2000 years ago, Hippocrates already highlighted ''*Let food be your medicine and medicine be your food*''. Various nutrients in the diet play a crucial role in maintaining an ''optimal'' immune response, such that deficient or excessive intakes can have negative consequences on the organism's immune status and susceptibility to a variety of pathologies. Over the last few decades, various immune-modulating nutrients have been identified, which interfere with IL6 gene expression. Currently, a broad range of phyto-pharmaceuticals with a claimed hormonal activity, called ''phyto-estrogens'', is recommended for prevention of various diseases related to a disturbed hormonal balance (i.e. menopausal ailments and/or prostate/breast cancer). In this respect, there is a renewed interest in soy isoflavones (genistein, daidzein, biochanin) as potential superior alternatives to the synthetic selective estrogen receptor modulators (SERMs), which are currently applied in hormone replacement therapy (HRT). As phyto-chemicals integrate hormonal ligand activities and interference with signaling cascades, therapeutic use may not be restricted to hormonal ailments only, but may have applications in cancer chemoprevention and/or NF-κB-related inflammatory disorders as well.
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Keywords: Phyto-estrogen; Immunity; Cancer; Hormone replacement therapy; NF-κB; IL6

1. Soy isoflavone phyto-pharmaceuticals

Numerous botanical species and plant parts contain a diverse array of polyphenolic non-steroidal phyto-chemicals that are synthesized as floral pigments for the attraction of insect pollinators and as signal molecules for beneficial micro-organisms in the rhizosphere. Besides these functions, they also have a role as anti-microbial/fungal defense compounds, and furthermore afford photoprotection [1].

Based on their chemical structure, phenolic phyto-estrogens can be classified into four main groups: isoflavonoids, flavonoids, stilbenes and lignans [2] (Fig. 1). Nowadays, many researchers focus on isoflavones, which are considered widespread in the plant kingdom, as these phyto-estrogens are mainly found in ''Leguminosae'' [3]. Within this specific plant family, they are especially abundant in soybean: approximately 2 g of isoflavones can be found per

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor (NR3A); ERR, estrogen-related receptor (NR3B); HRT, hormone replacement therapy; IκB, inhibitor of κB; IKK, IκB kinase; IL6, interleukin-6; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PPAR, peroxisome proliferator-activated receptor (NR1C); ROS, reactive oxygen species; SERM, selective estrogen receptor modulator

* Corresponding author. Tel.: +32 9 264 51 66; fax: +32 9 264 53 04.
E-mail address: guy.haegeman@ugent.be (G. Haegeman).

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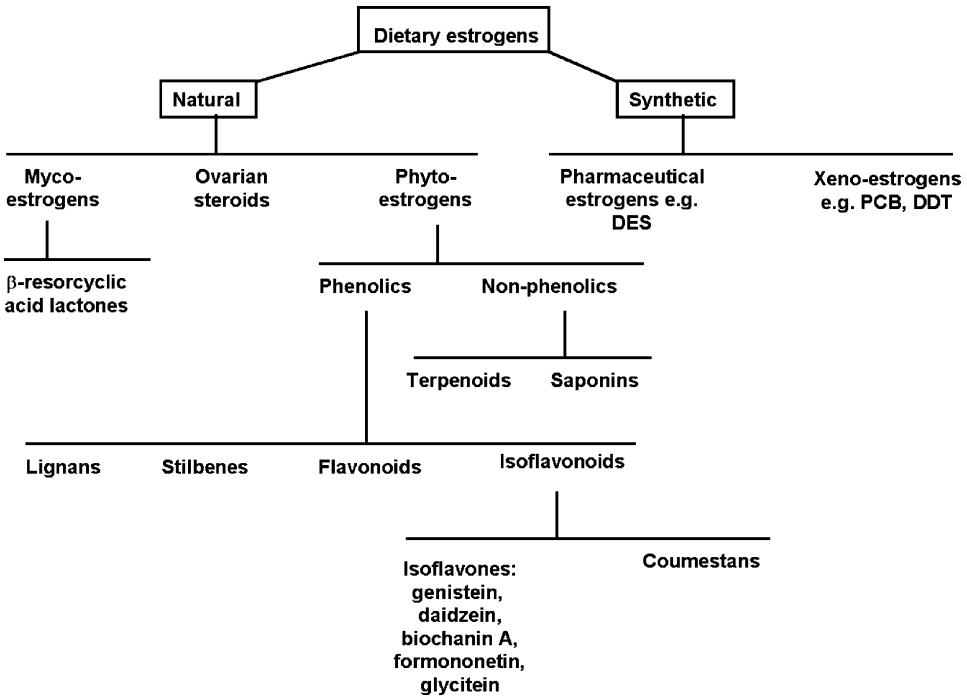

Fig. 1. Classes of (phyto-)estrogens (adapted from [2]).

kilogram fresh weight [4]. Among these isoflavones, genistein (4′,5,7-trihydroxyisoflavone), daidzein (4,7-dihydroxyisoflavone) and their 4′-methylether derivatives, biochanin A and formononetin, respectively, are the best known and widely available as herbal tablets in drug stores and health shops. They gained enormous attention as they were found to be appropriate ligands of the ER. Furthermore, structure–function studies have revealed that a stable, strong binding to the ER, and thus also the biological activity of 17β-estradiol, largely depends on the distance of approximately 11.5 Å between two well-defined hydroxyl groups. One of these is positioned in the A-ring of estrogen's steroid nucleus and the other at C(17) [5,6]. Because of the non-steroidal skeleton of isoflavones and their different spacial structure, phyto-estrogens when bound to the ER, were expected to act totally differently. Nevertheless, these substances share structural features with estrogen, in the sense that the presence of particular hydroxyl groups that can be positioned in a stereochemical alignment is virtually identical to the one of estrogen. Only lately, many studies have been undertaken to unveil the mode by which phyto-estrogens mediate their estrogenic effects. Assumptions range from mimicking normal estrogenic actions to competitive inhibitory effects. Crystallization of liganded receptors unambiguously reveals changes in ER conformation (e.g. helix 12 positioning) upon binding of classic estrogens, as compared to phyto-estrogens, which may already suggest ligand-selective and thus cofactor-dependent activities [7–16]. Notably, phyto-estrogens seem to preferentially bind the ERβ isoform, whereas classic estrogen exerts its effects via both receptors ERα and ERβ [17,18]. As such, they may act as natural SERMs, that elicit distinct therapeutical effects by selectively recruiting coregulatory factors to ERβ, which then specifically affect transcription [12,17,19–22]. Importantly, although the relative binding affinity of estrogen for each isotype receptor is stated to be at least 1000-fold higher than that of phyto-estrogens [18], the latter could evoke biological responses, because of the higher achievable concentrations in plasma as compared to the endogenous hormone estradiol [8,23]. Furthermore, although phyto-estrogens appear to have a greater affinity for ERβ than for ERα, they have a greater ERα-selective efficacy [24,25].

Although having specified functions in plants, most of the actions of phyto-estrogens seem to be different in humans, except for their reported anti-photodamaging quality. By this protection against excessive light, they prevent human skin carcinogenesis and ageing [26]. Overall, phyto-estrogens are presumed to complement and/or overlap in activities [27]. Apart from their estrogenic properties, other features could also be involved in their observed diverse action mechanisms, including binding to other nuclear receptors (ERR, PPAR, AhR, etc.), antioxidant effects due to their polyphenolic nature, modulation of steroid metabolism or of detoxification enzymes, interference with Ca-transport and/or Na^+/K^+ ATPases, or favorable effects on lipid and lipoprotein profiles [2,23,28–35]. In addition, genistein behaves as a broad specificity tyrosine protein kinase inhibitor [36] and is, furthermore, able to block the cell cycle in the G_2/M transition [37,38], to inhibit Akt kinase [39–41], topoisomerase II [42,43] and cAMP-phosphodiesterase-4 enzymes [44]. The presumable strength of phyto-estrogens could rely on the unique

	ER ligand	tyrosine kinase inhibitor	anti-oxidant	phosphodiesterase inhibitor	topoisomerase II inhibitor	cell cycle inhibitor
genistein	+	+	++	PDE4	+	G2/M
biochanin A	+	-	+	PDE4	-	G0/G1
daidzein	+	-	+	PDE1/3	-	G1/S

Fig. 2. Features of (phyto-)estrogens.

combination of several features with different potencies in one natural molecule (Fig. 2). Briefly, these characteristics may account for their potential favorable role in health and disease [45–48]. Considering the pleiotropic actions of phyto-estrogens, they may rather be reconsidered more generally as ''nutraceuticals''. This term is coined from nutrition and pharmaceuticals, defining any food substance which provides health benefits, including the prevention and treatment of disease [49]. Such substances may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products, and processed foods such as cereals, soups and beverages.

2. Health-beneficial effects of isoflavones

Impetus to the scientific inquiry into phyto-pharmaceutical effects is given by the epidemiological observation of striking historical and/or ethnic differences in hormone-related cancer rates and menopausal ailments [47,50,51]. The highest incidences of these cancers, menopausal symptoms as well as cardiovascular diseases are typically found in populations with western lifestyle, which include relatively high fat, meat-based and low-fiber diets [52]. Asian populations, however, with eastern habits, who consume plant-based diets, rich in phyto-estrogens, are less susceptible [53]. Moreover, the low cancer risk of Asian people migrating to the US rises to the American rates either generally in the same (e.g. prostate cancer) or for some cancer types in the next generation (e.g. breast cancer) [54,55]. This observation indicates that diet-related factors, rather than pure genetic factors, largely contribute to the occurrence of these pathophysiologies [50,56–59]. Accordingly, intense investigations point out the important role of consumption of dietary soy as the major source of isoflavones in the low cancer incidence [60,61]. Particular Japanese population groups are estimated to reach the highest intake of soy products, with levels up to 200 mg/day. Generally, the Asian consumption of legumes is assumed to supply 20–50 mg of isoflavones in the daily diet, which sharply contrasts with the western negligible amount of less than 1 mg isoflavones/day [62]. Consistent with the epidemiological studies are the findings that soy phyto-estrogens improve bone mass in peri- and postmenopausal women [63,64] and may decrease symptomology of menopause, such as hot flashes [65,66]. Furthermore, they prevent atherosclerosis of coronary arteries in monkeys [67] and significantly reduce cholesterol levels in hypercholesterolemic subjects [68]. In vitro studies indicate that phyto-estrogens inhibit proliferation of several tumours, induce apoptosis as well as differentiation, and reduce angiogenesis [69,70]. Moreover, soy seems to delay and protect against chemically induced mammary tumour formation in rodents [71,72]. In addition, some reports propose efficacious protection against breast cancer if soy intake is spread over lifetime, although the period before puberty and during adolescence may be of particular importance [73–75]. Hence, isoflavones as well as other classes of phyto-estrogens have been considered to exert anti-carcinogenic properties, and this mainly through anti-aromatase, anti-proliferative and anti-angiogenic mechanisms [29].

In many studies, the experimental concentrations, administered to cells or tissues, at which chemoprevention by phyto-estrogens is achieved are considered supraphysiological, since these might not be attained in case of phyto-estrogen supplementation to the diet. However, in a real life diet, dose responses of nutraceutical mixtures may reveal synergistic effects at more physiological doses [76–79]. In addition, chaperone protection and/or effective metabolization of these compounds may be less efficient in tissue culture conditions [80].

Isoflavones are exclusively occurring in plants as inactive glycosides (genistin, daidzin) or their methylated counterparts [81]. Once ingested, the absorption of these compounds requires initial hydrolysis of the sugar moiety or demethylation, respectively, by gut/bacteria-released intestinal enzymes in the digestive tract. After presumed non-ionic passive diffusion through the gut wall, they undergo extensive enterohepatic circulation. During this passage in the liver they are readily reconjugated to glucuronic acid and to a lesser extent to sulphate. Hence, only a small portion (less than 3%) of the free aglycone can been detected in blood. Moreover, the grade of further metabolization of isoflavones (to equol and *O*-desmethylangolensin for daidzein and to 2-(4-hydroxyphenyl)propanoic acid and tri-OH-benzene for genistein) by gut bacteria is subjected to large interindividual variation, depending on gastrointestinal microflora and diet [82] (Fig. 3). As phyto-estrogens can exist in presumably many distinct forms with totally different activities, the quest for

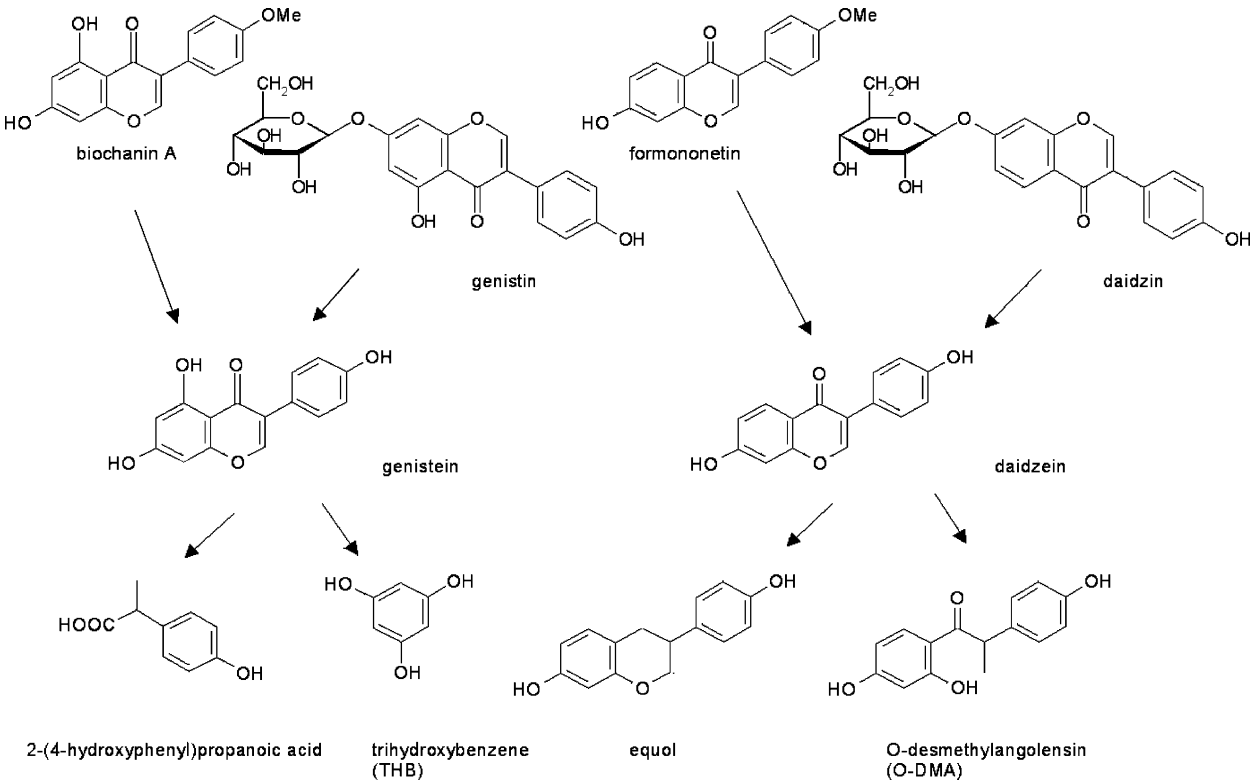

Fig. 3. Metabolization of isoflavones (adapted from [2]).

new metabolites and elucidating the total active plasma/tissue concentration of phyto-estrogens, their degradation pathways and clearance rates intensively continues [80,81]. Before undertaking dietary intervention trials, their pharmacokinetics and bioavailability in different tissues or target cells must be assessed carefully in order to make recommendations regarding long-term efficacy. Some measurements determining the plasma half-life of daidzein and genistein reveal that they occur approximately 8 h in adults, with peak concentrations at 6–8 h after administration of the pure compounds [83]. This strongly contrasts with the pharmacokinetic behaviour of environmental xeno-estrogens (e.g. PAHs, DDT, PCBs), which bioaccumulate and persist in fat tissues for years, because of their extremely long half-lives. This difference may partly explain the potential dangers of synthetic xeno-estrogens as endocrine disruptors [23,84]. They are reported to be linked to feminization of wildlife (fish, reptiles) as well as to the increasing incidence of breast and testicular cancers in humans [67,85,86].

All together, when viewed in its entirety, the current literature supports the safety of isoflavones as typically consumed in diets based on soy products [25,28,31,45,48,87–89]. Despite the putative inherent health benefits of bioactive phyto-chemicals, it is obvious that we still need to increase our knowledge of their molecular mechanisms, (long-term) safety, toxicity, dosage and efficacy prior to general application in cancer or hormone therapies [25,87]. Hence, strengthening our still limited understanding of the factors involved in their absorption and metabolism, including the role of intestinal microflora, may well be important in determining their clinical efficacy [87,88,90–92].

In further paragraphs, we will discuss more particularly possible actions of nutraceuticals in IL6 affections with respect to hormone receptor and NF-κB-dependent regulatory mechanisms.

3. IL6 physiology and pathology

The process of an inflammatory response to invading pathogens or damaging insults is of critical importance to the homeostasis of the human body. As this physiological inflammatory reaction, based on innate or adaptive immunity, determines health or illness, it needs to be precisely controlled by various feedback mechanisms [93–96]. As such, balanced inflammation consequently allows acute timely effects but no accelerated overreaction or chronic responses. Furthermore, ageing-related immune senescence and endocrinological changes were found to increase the risk of cardiovascular diseases, arthritis, type 2 diabetes, menopausal symptoms (e.g. hot flushes, vaginal dryness, osteoporosis, etc.), certain cancers and dementia, as a consequence of a tilted endogenous balance (Fig. 4).

The pleiotropic cytokine IL6 is a central player in immune homeostasis and affects inflammatory reactions, acute phase response, hematopoiesis, bone metabolism,

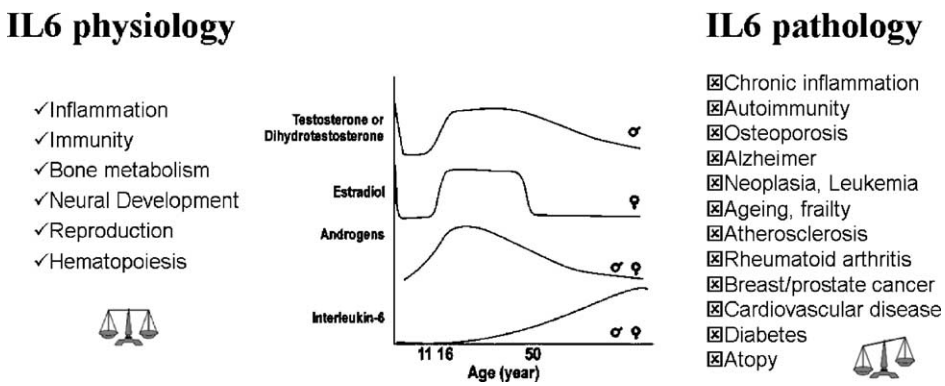

Fig. 4. IL6 gene expression and ageing (adapted from [94]).

reproduction (menstrual cycle, spermatogenesis) and ageing frailty [94–96]. Aberrant IL6 gene expression, however, has been associated with various chronic inflammatory disorders and ageing discomforts [96–98]. In addition to regulating inflammatory responses, IL6 modulates the growth of many tumour cells. Autocrine IL6-mediated signaling pathways have been implicated in tumour progression and chemoresistance in solid and hematopoietic tumours [99,100]. Tumorigenic activities of IL6 have also been described by switching TGF-β from a growth suppressing to a mitogenic factor [101] by affecting hormone responsiveness (i.e. ER, AR, etc.), hormone metabolism and synthesis (aromatase activity) [102–107], or translational upregulation of the apoptosis inhibitor XIAP [108]. Underscoring the potential value of targeted anti-IL6 therapy in cancer, a recent investigation showed that an anti-IL6 monoclonal antibody induces apoptosis and regression of xenografted human prostate cancer cells in a nude mouse model [109]. Accordingly, serum IL6 levels are currently considered a diagnostic marker for tumour progression, metastasis and prognosis in multiple cancer types (breast, prostate, lymphoma, lung, ovarian, gut and renal cell carcinoma) [100,106,110]. Nevertheless, the physiological activity of IL6 is complex, producing both pro-inflammatory and anti-inflammatory effects in the immune system [100]. It is therefore unclear whether elevated serum levels of IL6 are a consequence of or a contributory cause to advanced tumour stage [111–113]. Hence, the question as to whether the inflammatory infiltrate helps or hinders tumours is still open [114,115]. The described contrasting effects of IL6 include either a direct enhancement of auto- and paracrine-mediated tumour growth, or an anti-tumour effect by enhancement of the immune response (differentiation and maturation of B-cells, T-cells, dendritic cells, macrophages) and inhibition of tumour cell proliferation.

The menopause/andropause-associated disturbed hormonal balance is constituted by a remarkable rise of the IL6 expression level, while a rapid decline in circulating sex hormones (estrogen, androgen) is observed [94,116–119]. A general mechanism which is able to explain all consequences of the menopause on the metabolism of distinct organs (bone, blood vessels, brains, vagina, adipose tissue) seems unlikely. However, the age-related changes in the activity of proinflammatory cytokines (e.g. IL6) are beginning to emerge as a common theme that may have a significant impact on the function of all these body parts [94,96,117,120] (Fig. 4). Endogenous sex hormones should be considered valuable, as they have the ability to downregulate IL6 gene expression. Therefore, restoring the normal hormone level by HRT is considered an attractive option in the treatment of menopausal discomforts, although the risks/benefits of HRT are still contentious issues [28,87,121–128]. Recent investigations on the long-term effects of conventional hormone therapy with synthetic estrogen have demonstrated a substantially elevated risk of thrombosis and incidence of breast, endometrial and ovarian cancer [125,126,129]. Even synthetic SERMs, which were previously assumed to have a safer profile because of their tissue-specific ER agonist or antagonist activity, fell short of the general expectations. Tamoxifen, currently approved, recommended and applied in the treatment of ER-expressing breast cancer [130], behaves as an ER antagonist in breast tissue, whereas its observed agonist function in the uterus can lead to endometrium cancer [20]. In contrast, raloxifene is not proven to elevate the risk of certain estrogen-dependent cancers and it has recently been approved and predominantly been used for its potential preventive and therapeutic effect on osteoporosis [124]. Unfortunately, there are several indications that this SERM may not alleviate other menopause-associated problems. Moreover, it is believed to increase hot flashes [128]. In this respect, naturally occurring phyto-estrogens receive renewed attention as potentially superior alternatives to HRT, in view of the traditional and still continuing usage of plant extracts [28,31,48,89,117,122,123,131]. During evolution, the hormone metabolism has evolved to deal with endogenous hormone and exogenous dietary phyto-chemical exposure, but may be less well adapted to effectively

handle the synthetic chemicals of the 20th and 21st centuries [23].

4. NF-κB at the interface of inflammation and tumorigenesis: a paradigm for phyto-pharmaceuticals

A key player in IL6 gene expression is the transcription factor NF-κB, of which the transcriptional activity is regulated at multiple levels [99,132–135]. The NF-κB/IκB family of transcriptional regulators modulate the expression of a few hundred target genes, the majority of which participate in host immune responses [136–139]. Gene knock-out and other studies not only establish roles for NF-κB in the ontogeny of the immune system, but also demonstrate that NF-κB is responsible for various inflammatory disorders [140–142] and participates at multiple steps during oncogenesis and regulation of programmed cell death [143–145]. The mechanisms of constitutive NF-κB activation in tumours and lymphoma start to be unraveled and blockade of NF-κB levels is demonstrated to result in suppression of angiogenesis, invasion, metastasis and therapy resistance [146–150].

Because of its pivotal role in numerous essential cell system processes, NF-κB has become the focal point for intense drug discovery or drug development efforts, as well as the first target for innovative therapies of various European and American pharmaceutical companies [151]. Consequently, many investigations have been conducted to explore the upstream signaling pathways that lead to NF-κB activation. Several of these signaling molecules can serve as potential pharmaceutical targets for specific blocking the NF-κB activity so as to interrupt disease processes [152]. Since many of these proteins transmit more than one signal to downstream targets, the transmission of signals towards NF-κB is suggested to involve a network, rather than a linear sequence [135,153]. In addition, functional redundancy of signal molecules may further entail experimental constraints to elucidate NF-κB's upstream partners and evaluate their sensitivity to particular phyto-chemicals. This latter remains a hot topic, since the use of plant compounds as templates would allow the construction of novel pharmacological drugs, with enhanced biological properties. This implies that these drugs have to be highly suitable in selectively modulating NF-κB activity in specific pathophysiological conditions, which are caused by deregulation of intracellular cascades [154]. Nowadays, the Holy Grail of many pharmaceutical companies has rather shifted from random exploration of synthetic compound libraries to the identification of lead compounds in medicinal plant extracts with potential NF-κB-modulating activity [131,155]. Yet, a greater knowledge of the molecular determinants used by these compounds to interfere with the NF-κB pathway should provide clues for the development of more specific and efficacious NF-κB inhibitors [151]. Sofar, the cytoplasmic regulatory event in which NF-κB is released from its physiological inhibitor, the IκB complex, has gained considerable attention [154,156]. However, the strategy of blocking this process has the important disadvantage of causing detrimental side effects, as NF-κB is also a crucial component of the immune system. Therefore, anti-inflammatory therapies may focus on less aggressive treatments, which interfere without affecting DNA binding after NF-κB release. To this purpose, a valuable alternative may be the NF-κB/chromatine interface in the nucleus, where interactions of NF-κB with particular cofactors or defined nucleosomes could be influenced [157–162]. Unraveling the relationship between chromatin and NF-κB regulation may reveal new targets for the design of innovative inhibitors. As chromatine-embedded promoter enhanceosomes behave as sophisticated protein modules, receptive to various signals, prospective therapies may benefit from combined structural (selective ligands) and signaling (selective inhibitors) approaches to establish effective but harmless treatments [163–168].

Interestingly, we and others obtained compelling evidence indicating that phyto-estrogens (isoflavones) have a potential NF-κB-inhibitory activity and/or inhibitory effects on targets of the inflammatory/apoptotic cascade [29,40,41,81,169,170] (*). More particularly, therapeutic effects of isoflavones in NF-κB-dependent inflammation have been observed in asthma [171], arthritis [172] and lung injury [173]. In addition, chemopreventive effects of genistein on NF-κB-dependent apoptotic responses have been described in lymphoma [174] and breast [39,41,55,175], prostate [40,176] or pancreas [177,178] cancer.

The mechanisms of ER-mediated NF-κB inhibition are cell type-dependent [179–182] and may involve direct protein–protein interactions [183], inhibition of DNA binding [184,185], induction of IκB expression [186] or limited coactivator sharing [187]. Apart from ER [183,184,188–190], reciprocal transcriptional modulation between NF-κB and several other nuclear hormone receptors has been reported as well, including PPARα [191], PPARγ [192], GR (glucocorticoid receptor; NR3C1) [193,194], AR [195,196], PR (progesterone receptor; NR3C3) [197,198], AhR [199], LXR (liver X receptor; NR1H3) [200] and vitamin D receptor (NR1I1) [201,202]. Interestingly, as ERβ was stated to be more potent in transcriptional repression of NF-κB than ERα and as phyto-estrogens preferentially bind to ERβ, NF-κB-dependent gene regulation may be differentially affected by synthetic estrogens as compared to phyto-chemicals [16,17,180,203]. In addition, since phyto-estrogen-mediated induction of PPARγ [204], AhR [205], vitamin D receptor and ERR responses [206] has also been observed, the complexity of phyto-chemical hormone cross-talk with NF-κB is further augmented [9,23].

* Vanden Berghe et al., in preparation.

Further evidence indicates that hormones and kinases such as IKK, PI3K, Akt and MAPK are key elements in intracellular signal cascades regulating NF-κB and/or hormone (ER, AR (androgen receptor; NR3C4), AhR, PPAR, etc.) activity [132,133,207]. Moreover, some reports assert the existence of various levels of ''cross-talk'', exemplified by the progress of breast cancer [208]. An initial hormone-dependent, anti-estrogen-sensitive, non-metastatic cancer often changes its phenotype towards a hormone-independent, endocrine therapy-resistant form with highly invasive growth properties upon transition from inducible to constitutive receptor tyrosine kinase (RTK)/MAPK signaling [209,210]. The exact mechanisms for either intrinsic or acquired resistance are not yet completely resolved, although they clearly include ER-coregulatory factors and functional interdependence between ER, NF-κB, growth factors and kinase networks [207,208,211–217]. Interestingly, as estrogen effects have also been observed in ER-negative breast cancer cells (SK-BR3), a growing body of evidence suggests that not every estrogen effect can be explained by the classic genomic model of hormone action, which involves (in)direct DNA binding of the estrogen receptor (ER) on its target genes [218]. Of special note, genomic (phyto-)estrogen actions have now been extended by more rapid nongenomic actions on signaling pathways [219–221], depending on the presence of ER-membrane receptors [216,222], (phyto-)estrogen interactions with G protein coupled receptors (GPR30) [218,223–225] or the laminin receptor [226]. Further studies are required to determine relative contribution of genomic and nongenomic actions in NF-κB transrepression by (phyto-)estrogens.

With respect to tyrosine kinase inhibitor activities of isoflavones [36], involvement of tyrosine phosphorylation [227,228] has been demonstrated in the signaling cascade of TNF [229–232], Toll-like receptor (TLR) [233] and growth factor signaling (EGF, Her/neu) [210,234–237]. Subsequent inhibition by phyto-estrogens [238,239] decreases further downstream signaling pathways (Akt, IKK, MAPK, etc.) [170,240–243].

Finally, ROS, which comprise hydrogen peroxide, hydroxyl radicals and superoxide anions, have been postulated to act as mitochondria-derived second messengers leading to NF-κB activation [244–247]. Yet, this role of ROS has been a contentious issue for many years [248]. In accordance with ROS as signaling intermediates, the activation of NF-κB by different agents (TNF, IL1, PMA, etc.) can be prevented by anti-oxidantia and by chelation of iron ions, which are required to convert oxygen intermediates to the highly reactive hydroxyl radicals [249]. However, the remarkable finding that anti-oxidantia reduce ligand-receptor affinity (e.g. TNF/TNFR), and thus lower the magnitude of receptor signaling disputes this hypothesis [250,251]. In another study, NF-κB is crucial to eliminate TNF-induced ROS accumulation in mouse embryonal fibroblasts to lower prolonged MAPK activation and inhibit cell death [252]. Whether phyto-estrogens with anti-oxidative characteristics would affect NF-κB signaling in either of these two ways is still an unresolved issue [253].

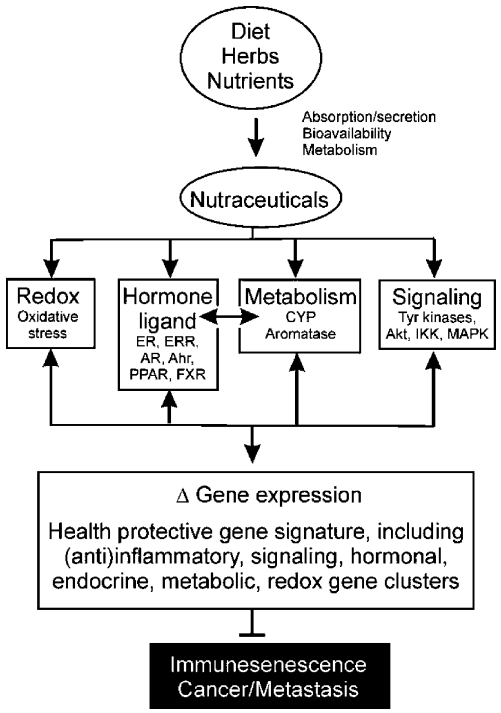

Fig. 5. Pleiotropic activities of nutraceuticals.

Considering the pleiotropic activities of nutraceuticals, it would certainly be very interesting to evaluate which activity predominates in their NF-κB modulation within a gene- or cell-specific context, as compared to classical estrogens. Further profound structure–function analysis of phyto-chemicals may allow to define core structure elements that account for some of their subactivities. At this stage, it may be too reductionistic to explain all chemopreventive effects of phyto-estrogens in breast cancer by its natural SERM properties only (Fig. 5). Upon investigation of raw array data sets of 78 breast cancer patients with good (44) or bad (34) prognosis signature [254,255] (data sets freely available at http://www.rii.com/publications/2002/vantveer.htm), many significant changes can be observed in hormonal (ERα, AhR, PR, PPARγ), oxidative (HIF1a, SOD2, GSTM3, HSF2BP, HSPA2, HSPA6), metabolic (CYP1B1, CYP7B1, CYP26A1), and signaling (IL6, IL8, VEGF) pathways. This discrepancy reflects distinct transcriptome circuitries in both patient populations which may not exclusively depend on estrogenic signaling parameters. In this respect, it would be interesting to compare how soy isoflavones versus synthetic estradiol and SERMS (tamoxifen, raloxifen) affect gene expression patterns of a good/poor prognosis signature in breast cancer cells [256].

5. IL6 gene regulation, HRT and "inflamm-ageing"

Transcription and expression of the multifunctional IL6 cytokine gene is tightly regulated at the level of its pro-

moter, which acts as a sophisticated biosensor for environmental stress, thus controlling the above-described immunological homeostasis [99]. IL6 is normally expressed at low levels, except during infection, trauma, ageing or other stress conditions, upon which it can be secreted by various cell types, including endothelial cells, B-cells, T-cells, macrophages, dendritic cells, and tumour cells [100]. Among several factors that can downregulate IL6 gene expression are hormones (corticosteroids, sex hormones, etc.) by endocrinological feedback mechanisms [93,257–260] and antioxidants [98,244]. Characterization of the human IL6 promoter revealed a highly conserved control region of 300 bp upstream of the transcriptional initiation site, which contains NF-κB, AP1, CREB and C/EBP regulatory elements. These responsive sites are necessary for IL6 gene induction by a variety of stimuli, commonly associated with acute inflammatory or proliferative states [99,157]. Recently, we have found that IL6 gene induction in response to inflammatory stress requires activation of the MAPK–MSK (mitogen- and stress-activated kinase) kinase pathway, which phosphorylates NF-κB p65 and histone H3, to establish a transcription-competent promoter complex (enhanceosome) [165,261,262]. Within this model, we have proposed various mechanisms to explain hormonal repression of NF-κB-driven genes, more particularly of the IL6 gene [191,194,263,264]. As such, the functional interaction between the ER and the proinflammatory transcription factor NF-κB, which regulates the IL6 promoter activity, has been suggested to play a key role in (phyto-)estrogen prevention of age-related symptoms during HRT [116–118,120,185,264]. However, taking into account the discouraging HRT clinical trials with synthetic hormones [125–128], it may be too simplistic to reverse ageing frailty by restoring physiological IL6 levels by hormone complementation alone. Elderly humans also have altered cellular redox levels which contribute in progression of chronic diseases of ageing. Poorly maintained cellular redox during ageing affects immune (NF-κB) and hormone receptor (ER) functions as well, besides hormonal imbalances [98,265–269]. Although HRT may indeed relieve some ageing discomforts, special attention should be focused as well on dysfunctional hormone receptors and immune senescence (alternatively called ''inflamm-ageing'') as a consequence of oxidative damage [270–277]. In this respect, isoflavone phyto-estrogens may be superior to classical synthetic estrogens in HRT in restoring immune physiology, since they may have a better ratio of anti-oxidant/estrogen potencies which may protect against immune senescence, in addition to complementation of hormone levels [98,253,278,279].

6. Nutrigenomics: the next frontier in nutraceutical therapy?

Progress in the battle against ageing disease and suffering is being accelerated by the availability of genomic information for humans, mice and other organisms. The interface between the nutritional environment and cellular/genetic processes is being referred to as ''nutrigenomics''. Nutrigenomics seeks to provide a molecular genetic understanding of how common dietary chemicals (i.e. nutrition) affect health by altering the expression and/or structure of an individual's genetic make-up. This in turn may alter disease initiation, development or progression as well. In this view, pharmaceutical companies are incorporating genotyping as part of their clinical trails to predict drug safety, toxicity and efficacy. Today, the concept of ''personalized'' medicine is being extended to the field of nutrition. The same tools and methods used in pharmacogenomics (single nucleotide polymorphisms (SNP) analysis, gene-expression profiling, proteomics, metabolomics and bioinformatics) are being used to examine an individual's response to his or her nutritional environment. The desired outcome of nutrigenomics is the use of personalized diets to delay the onset of disease, and optimize and maintain human health [256]. With the technical advances of high-throughput genomic tools to determine SNPs, we are nowadays more aware of the specific genes that can directly and indirectly contribute to individual differences in the susceptibility to carcinogenesis and/or drug metabolization. Once high-risk groups are identified, specific dietary supplements might be recommended for these individuals, whose disrupted cellular signaling can be restored or approved by this tailored supplementation [30].

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References

[1] Dixon RA, Steele CL. Flavonoids and isoflavonoids—a gold mine for metabolic engineering. Trends Plant Sci 1999;4:394–400.
[2] Cos P, De Bruyne T, Apers S, Vanden Berghe D, Pieters L, Vlietinck AJ. Phytoestrogens: recent developments. Planta Med 2003;69:589–99.
[3] King A, Young G. Characteristics and occurrence of phenolic phytochemicals. J Am Diet Assoc 1999;99:213–8.
[4] Reinli K, Block G. Phytoestrogen content of foods—a compendium of literature values. Nutr Cancer 1996;26:123–48.
[5] Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther 2002;96:67–202.
[6] Dixon RA, Ferreira D. Genistein. Phytochemistry 2002;60:205–11.
[7] Gustafsson JA. Therapeutic potential of selective estrogen receptor modulators. Curr Opin Chem Biol 1998;2:508–11.
[8] Gustafsson JA. What pharmacologists can learn from recent advances in estrogen signalling. Trends Pharmacol Sci 2003;24:479–85.

[9] Weihua Z, Andersson S, Cheng G, Simpson ER, Warner M, Gustafsson JA. Update on estrogen signaling. FEBS Lett 2003;546: 17–24.
[10] Metivier R, Stark A, Flouriot G, Hubner MR, Brand H, Penot G, et al. A dynamic structural model for estrogen receptor-alpha activation by ligands, emphasizing the role of interactions between distant A and E domains. Mol Cell 2002;10:1019–32.
[11] Benassayag C, Perrot-Applanat M, Ferre F. Phytoestrogens as modulators of steroid action in target cells. J Chromatogr B Anal Technol Biomed Life Sci 2002;777:233–48.
[12] Jordan VC. The secrets of selective estrogen receptor modulation: cell-specific coregulation. Cancer Cell 2002;1:215–7.
[13] Jordan VC, Schafer JM, Levenson AS, Liu H, Pease KM, Simons LA, et al. Molecular classification of estrogens. Cancer Res 2001;61:6619–23.
[14] Levenson AS, Svoboda KM, Pease KM, Kaiser SA, Chen B, Simons LA, et al. Gene expression profiles with activation of the estrogen receptor alpha-selective estrogen receptor modulator complex in breast cancer cells expressing wild-type estrogen receptor. Cancer Res 2002;62:4419–26.
[15] Dobrzycka KM, Townson SM, Jiang S, Oesterreich S. Estrogen receptor corepressors—a role in human breast cancer? Endocr Relat Cancer 2003;10:517–36.
[16] Nettles KW, Sun J, Radek JT, Sheng S, Rodriguez AL, Katzenellenbogen JA, et al. Allosteric control of ligand selectivity between estrogen receptors alpha and beta: implications for other nuclear receptors. Mol Cell 2004;13:317–27.
[17] An J, Tzagarakis-Foster C, Scharschmidt TC, Lomri N, Leitman DC. Estrogen receptor beta-selective transcriptional activity and recruitment of coregulators by phytoestrogens. J Biol Chem 2001;276:17808–14.
[18] Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139:4252–63.
[19] Enmark E, Gustafsson JA. Newly discovered estrogen receptor: new therapeutic possibilities in postmenopausal symptoms, osteoporosis, cancer of the breast and prostate. Lakartidningen 1998;95:1945–9.
[20] Katzenellenbogen BS, Katzenellenbogen JA. Biomedicine: defining the "S" in SERMs. Science 2002;295:2380–1.
[21] Sun J, Meyers MJ, Fink BE, Rajendran R, Katzenellenbogen JA, Katzenellenbogen BS. Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-alpha or estrogen receptor-beta. Endocrinology 1999;140:800–4.
[22] Shiau AK, Barstad D, Radek JT, Meyers MJ, Nettles KW, Katzenellenbogen BS, et al. Structural characterization of a subtype-selective ligand reveals a novel mode of estrogen receptor antagonism. Nat Struct Biol 2002;9:359–64.
[23] Jacobs MN, Lewis DF. Steroid hormone receptors and dietary ligands: a selected review. Proc Nutr Soc 2002;61:105–22.
[24] Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J, Nilsson S. Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. Mol Pharmacol 1998;54:105–12.
[25] Beck V, Unterrieder E, Krenn L, Kubelka W, Jungbauer A. Comparison of hormonal activity (estrogen, androgen and progestin) of standardized plant extracts for large scale use in hormone replacement therapy. J Steroid Biochem Mol Biol 2003;84:259–68.
[26] Wei H, Saladi R, Lu Y, Wang Y, Palep SR, Moore J, et al. Isoflavone genistein: photoprotection and clinical implications in dermatology. J Nutr 2003;133:3811S–9S.
[27] Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 2002;113(Suppl 9B):71S–88S.
[28] Bolego C, Poli A, Cignarella A, Paoletti R. Phytoestrogens: pharmacological and therapeutic perspectives. Curr Drug Targets 2003;4:77–87.
[29] Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003;3:768–80.
[30] Muller M, Kersten S. Nutrigenomics: goals and strategies. Nat Rev Genet 2003;4:315–22.
[31] Albertazzi P, Purdie D. The nature and utility of the phytoestrogens: a review of the evidence. Maturitas 2002;42:173–85.
[32] Ososki AL, Kennelly EJ. Phytoestrogens: a review of the present state of research. Phytother Res 2003;17:845–69.
[33] Muroya T, Ihara Y, Ikeda S, Yasuoka C, Miyahara Y, Urata Y, et al. Oxidative modulation of NF-kappaB signaling by oxidized low-density lipoprotein. Biochem Biophys Res Commun 2003;309:900–5.
[34] Naderi GA, Asgary S, Sarraf-Zadegan N, Shirvany H. Anti-oxidant effect of flavonoids on the susceptibility of LDL oxidation. Mol Cell Biochem 2003;246:193–6.
[35] Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. Antioxidant activity of phytoestrogenic isoflavones. Free Radic Res 1997;26:63–70.
[36] Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987;262:5592–5.
[37] Casagrande F, Darbon JM. Effects of structurally related flavonoids on cell cycle progression of human melanoma cells: regulation of cyclin-dependent kinases CDK2 and CDK1. Biochem Pharmacol 2001;61:1205–15.
[38] Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, Matsumoto K, et al. Genistein arrests cell cycle progression at G2-M. Cancer Res 1993;53:1328–31.
[39] Chinni SR, Alhasan SA, Multani AS, Pathak S, Sarkar FH. Pleotropic effects of genistein on MCF-7 breast cancer cells. Int J Mol Med 2003;12:29–34.
[40] Li Y, Sarkar FH. Inhibition of nuclear factor kappaB activation in PC3 cells by genistein is mediated via Akt signaling pathway. Clin Cancer Res 2002;8:2369–77.
[41] Gong L, Li Y, Nedeljkovic-Kurepa A, Sarkar FH. Inactivation of NF-kappaB by genistein is mediated via Akt signaling pathway in breast cancer cells. Oncogene 2003;22:4702–9.
[42] Salti GI, Grewal S, Mehta RR, Das Gupta TK, Boddie Jr AW, Constantinou AI. Genistein induces apoptosis and topoisomerase II-mediated DNA breakage in colon cancer cells. Eur J Cancer 2000;36:796–802.
[43] Markovits J, Linassier C, Fosse P, Couprie J, Pierre J, Jacquemin-Sablon A, et al. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res 1989;49:5111–7.
[44] Nichols MR, Morimoto BH. Differential inhibition of multiple cAMP phosphodiesterase isozymes by isoflavones and tyrphostins. Mol Pharmacol 2000;57:738–45.
[45] Sarkar FH, Li Y. Soy isoflavones and cancer prevention. Cancer Invest 2003;21:744–57.
[46] Messina MJ. Legumes and soybeans: overview of their nutritional profiles and health effects. Am J Clin Nutr 1999;70:439S–50S.
[47] Hughes CL, Dhiman TR. Dietary compounds in relation to dietary diversity and human health. J Med Food 2002;5:51–68.
[48] Wuttke W, Jarry H, Becker T, Schultens A, Christoffel V, Gorkow C, et al. Phytoestrogens: endocrine disrupters or replacement for hormone replacement therapy? Maturitas 2003;44(Suppl 1):S9–S20.
[49] Kalra EK. Nutraceutical—definition and introduction. AAPS Pharm Sci 2003;5:E25.
[50] Ziegler RG, Hoover RN, Pike MC, Hildesheim A, Nomura AM, West DW, et al. Migration patterns and breast cancer risk in Asian–American women. J Natl Cancer Inst 1993;85:1819–27.
[51] Bradlow HL, Sepkovic DW. Diet and breast cancer. Ann NY Acad Sci 2002;963:247–67.
[52] Bingham S, Riboli E. Diet and cancer—the European prospective investigation into cancer and nutrition. Nat Rev Cancer 2004;4: 206–15.

[53] Rosenberg Zand RS, Jenkins DJ, Diamandis EP. Flavonoids and steroid hormone-dependent cancers. J Chromatogr B Analyt Technol Biomed Life Sci 2002;777:219–32.
[54] Buell P. Changing incidence of breast cancer in Japanese–American women. J Natl Cancer Inst 1973;51:1479–83.
[55] Lamartiniere CA. Protection against breast cancer with genistein: a component of soy. Am J Clin Nutr 2000;71:1705S–7S (discussion 1708S-9S).
[56] Wu AH, Ziegler RG, Pike MC, Nomura AM, West DW, Kolonel LN, et al. Menstrual and reproductive factors and risk of breast cancer in Asian–Americans. Br J Cancer 1996;73:680–6.
[57] Parkin DM. Cancers of the breast, endometrium and ovary: geographic correlations. Eur J Cancer Clin Oncol 1989;25:1917–25.
[58] MacMahon B, Cole P, Brown J. Etiology of human breast cancer: a review. J Natl Cancer Inst 1973;50:21–42.
[59] Taioli E, Garte SJ, Trachman J, Garbers S, Sepkovic DW, Osborne MP, et al. Ethnic differences in estrogen metabolism in healthy women. J Natl Cancer Inst 1996;88:617.
[60] Birt DF, Hendrich S, Wang W. Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol Ther 2001;90:157–77.
[61] Adlercreutz H. Phyto-oestrogens and cancer. Lancet Oncol 2002;3:364–73.
[62] Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr 1998;128:209–13.
[63] Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman Jr JW. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 1998;68:1375S–9S.
[64] Alekel DL, Germain AS, Peterson CT, Hanson KB, Stewart JW, Toda T. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr 2000;72:844–52.
[65] Murkies AL, Lombard C, Strauss BJ, Wilcox G, Burger HG, Morton MS. Dietary flour supplementation decreases post-menopausal hot flushes: effect of soy and wheat. Maturitas 1995;21:189–95.
[66] Nagata C, Takatsuka N, Kawakami N, Shimizu H. Soy product intake and hot flashes in Japanese women: results from a community-based prospective study. Am J Epidemiol 2001;153:790–3.
[67] Anthony MS, Clarkson TB, Hughes Jr CL, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996;126:43–50.
[68] Crouse III JR, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999;159:2070–6.
[69] Messina MJ, Persky V, Setchell KD, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr Cancer 1994;21:113–31.
[70] Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, et al. Genistein, a dietary-derived inhibitor of in vitro angiogenesis. Proc Natl Acad Sci USA 1993;90:2690–4.
[71] Jin Z, MacDonald RS. Soy isoflavones increase latency of spontaneous mammary tumors in mice. J Nutr 2002;132:3186–90.
[72] Lamartiniere CA, Murrill WB, Manzolillo PA, Zhang JX, Barnes S, Zhang X, et al. Genistein alters the ontogeny of mammary gland development and protects against chemically-induced mammary cancer in rats. Proc Soc Exp Biol Med 1998;217:358–64.
[73] Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. Exposure of infants to phyto-oestrogens from soy-based infant formula. Lancet 1997;350:23–7.
[74] Colditz GA, Frazier AL. Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus. Cancer Epidemiol Biomarkers Prev 1995;4:567–71.
[75] Adlercreutz H. Phytoestrogens and breast cancer. J Steroid Biochem Mol Biol 2002;83:113–8.
[76] Rajapakse N, Silva E, Kortenkamp A. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ Health Persp 2002;110:917–21.
[77] Silva E, Rajapakse N, Kortenkamp A. Something from ''nothing''—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ Sci Technol 2002;36:1751–6.
[78] Kortenkamp A, Altenburger R. Synergisms with mixtures of xenoestrogens: a reevaluation using the method of isoboles. Sci Total Environ 1998;221:59–73.
[79] Kortenkamp A, Altenburger R. Approaches to assessing combination effects of oestrogenic environmental pollutants. Sci Total Environ 1999;233:131–40.
[80] Boersma BJ, Barnes S, Kirk M, Wang CC, Smith M, Kim H, et al. Soy isoflavonoids and cancer—metabolism at the target site. Mutat Res 2001;480–481:121–7.
[81] Kim BH, Chung EY, Ryu JC, Jung SH, Min KR, Kim Y. Anti-inflammatory mode of isoflavone glycoside sophoricoside by inhibition of interleukin-6 and cyclooxygenase-2 in inflammatory response. Arch Pharm Res 2003;26:306–11.
[82] Rowland I, Faughnan M, Hoey L, Wahala K, Williamson G, Cassidy A. Bioavailability of phyto-oestrogens. Br J Nutr 2003;89(Suppl 1):S45–58.
[83] Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. Am J Clin Nutr 1998;68:1453S–61S.
[84] Safe SH. Environmental and dietary estrogens and human health: is there a problem? Environ Health Persp 1995;103:346–51.
[85] Kelce WR, Wilson EM. Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications. J Mol Med 1997;75:198–207.
[86] Adlercreutz H. Phytoestrogens: epidemiology and a possible role in cancer protection. Environ Health Persp 1995;103(Suppl 7):103–12.
[87] Kronenberg F, Fugh-Berman A. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. Ann Intern Med 2002;137:805–13.
[88] Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. Obstet Gynecol 1996;87:897–904.
[89] Cranney A, Guyatt G, Griffith L, Wells G, Tugwell P, Rosen C. Meta-analyses of therapies for postmenopausal osteoporosis. IX. Summary of meta-analyses of therapies for postmenopausal osteoporosis. Endocrinol Rev 2002;23:570–8.
[90] Ewies AA. Phytoestrogens in the management of the menopause: up-to-date. Obstet Gynecol Surv 2002;57:306–13.
[91] Stark A, Madar Z. Phytoestrogens: a review of recent findings. J Pediatr Endocrinol Metab 2002;15:561–72.
[92] Russell L, Hicks GS, Low AK, Shepherd JM, Brown CA. Phytoestrogens: a viable option? Am J Med Sci 2002;324:185–8.
[93] Kariagina A, Romanenko D, Ren SG, Chesnokova V. Hypothalamic-pituitary cytokine network. Endocrinology 2004;145:104–12.
[94] Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med 1998;128:127–37.
[95] Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002;13:357–68.
[96] Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000;51:245–70.
[97] Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol 1993;54:1–78.
[98] Zhang J, Dai J, Lu Y, Yao Z, O'Brien CA, Murtha JM, et al. In vivo visualization of aging-associated gene transcription:

evidence for free radical theory of aging. Exp Gerontol 2004;39: 239–47.
[99] Vanden Berghe W, Vermeulen L, De Wilde G, De Bosscher K, Boone E, Haegeman G. Signal transduction by tumor necrosis factor and gene regulation of the inflammatory cytokine interleukin-6. Biochem Pharmacol 2000;60:1185–95.
[100] Trikha M, Corringham R, Klein B, Rossi JF. Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. Clin Cancer Res 2003;9:4653–65.
[101] Park JI, Lee MG, Cho K, Park BJ, Chae KS, Byun DS, et al. Transforming growth factor-beta1 activates interleukin-6 expression in prostate cancer cells through the synergistic collaboration of the Smad2, p38-NF-kappaB, JNK, and Ras signaling pathways. Oncogene 2003;22:4314–32.
[102] Jia L, Choong CS, Ricciardelli C, Kim J, Tilley WD, Coetzee GA. Androgen receptor signaling: mechanism of interleukin-6 inhibition. Cancer Res 2004;64:2619–26.
[103] Bachelot T, Ray-Coquard I, Menetrier-Caux C, Rastkha M, Duc A, Blay JY. Prognostic value of serum levels of interleukin-6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients. Br J Cancer 2003;88:1721–6.
[104] Honma S, Shimodaira K, Shimizu Y, Tsuchiya N, Saito H, Yanaihara T, et al. The influence of inflammatory cytokines on estrogen production and cell proliferation in human breast cancer cells. Endocrinol J 2002;49:371–7.
[105] Purohit A, Newman SP, Reed MJ. The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer. Breast Cancer Res 2002;4:65–9.
[106] Smith PC, Hobisch A, Lin DL, Culig Z, Keller ET. Interleukin-6 and prostate cancer progression. Cytokine Growth Factor Rev 2001;12:33–40.
[107] Lin DL, Whitney MC, Yao Z, Keller ET. Interleukin-6 induces androgen responsiveness in prostate cancer cells through up-regulation of androgen receptor expression. Clin Cancer Res 2001;7: 1773–81.
[108] Yamagiwa Y, Marienfeld C, Meng F, Holcik M, Patel T. Translational regulation of x-linked inhibitor of apoptosis protein by interleukin-6: a novel mechanism of tumor cell survival. Cancer Res 2004;64:1293–8.
[109] Smith PC, Keller ET. Anti-interleukin-6 monoclonal antibody induces regression of human prostate cancer xenografts in nude mice. Prostate 2001;48:47–53.
[110] Zhang GJ, Adachi I. Serum interleukin-6 levels correlate to tumor progression and prognosis in metastatic breast carcinoma. AntiCancer Res 1999;19:1427–32.
[111] DeMichele A, Martin AM, Mick R, Gor P, Wray L, Klein-Cabral M, et al. Interleukin-6-174G→C polymorphism is associated with improved outcome in high-risk breast cancer. Cancer Res 2003;63:8051–6.
[112] Iacopetta B, Grieu F, Joseph D. The −174 G/C gene polymorphism in interleukin-6 is associated with an aggressive breast cancer phenotype. Br J Cancer 2004;90:419–22.
[113] Salgado R, Junius S, Benoy I, Van Dam P, Vermeulen P, Van Marck E, et al. Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. Int J Cancer 2003;103:642–6.
[114] Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–45.
[115] Brigati C, Noonan DM, Albini A, Benelli R. Tumors and inflammatory infiltrates: friends or foes? Clin Exp Metastas 2002;19:247–58.
[116] Burger HG, Dudley EC, Robertson DM, Dennerstein L. Hormonal changes in the menopause transition. Recent Prog Horm Res 2002;57:257–75.
[117] Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. Endocrinol Rev 2002;23:90–119.
[118] Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, Glaser R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. Proc Natl Acad Sci USA 2003;100:9090–5.
[119] Lamberts SWJ. Endocrinology and aging. Williams Textbook of Endocrinology. 2002. p. 1287–1302.
[120] Ershler WB, Harman SM, Keller ET. Immunologic aspects of osteoporosis. Dev Comp Immunol 1997;21:487–99.
[121] Wuttke W, Jarry H, Westphalen S, Christoffel V, Seidlova-Wuttke D. Phytoestrogens for hormone replacement therapy? J Steroid Biochem Mol Biol 2002;83:133–47.
[122] Wells G, Tugwell P, Shea B, Guyatt G, Peterson J, Zytaruk N, et al. Meta-analyses of therapies for postmenopausal osteoporosis. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. Endocrinol Rev 2002;23:529–39.
[123] Marsden J. The menopause, hormone replacement therapy and breast cancer. J Steroid Biochem Mol Biol 2002;83:123–32.
[124] Guyatt GH, Cranney A, Griffith L, Walter S, Krolicki N, Favus M, Rosen C. Summary of meta-analyses of therapies for postmenopausal osteoporosis and the relationship between bone density and fractures. Endocrinol Metab Clin North Am 2002;31:659–679, xii.
[125] Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 2003;362:419–27.
[126] Hulley SB, Grady D. The WHI estrogen-alone trial—do things look any better? J Am Med Assoc 2004;291:1769–71.
[127] Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. J Am Med Assoc 2002;288:321–33.
[128] Nelson HD. Commonly used types of postmenopausal estrogen for treatment of hot flashes: scientific review. J Am Med Assoc 2004;291:1610–20.
[129] Noller KL. Estrogen replacement therapy and risk of ovarian cancer. J Am Med Assoc 2002;288:368–9.
[130] Jordan VC. Tamoxifen: a most unlikely pioneering medicine. Nat Rev Drug Discov 2003;2:205–13.
[131] Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Cancer 2002;2:143–8.
[132] Richmond A. Nf-kappab, chemokine gene transcription and tumour growth. Nat Rev Immunol 2002;2:664–74.
[133] Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol 2002;2:725–34.
[134] Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999;18:6853–66.
[135] Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, et al. A physical and functional map of the human TNF-alpha/NF-kappaB signal transduction pathway. Nat Cell Biol 2004;6:97–105.
[136] Ghosh S, May MJ, Kopp EB. NF-kappaB and rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 1998;16:225–60.
[137] Zhou A, Scoggin S, Gaynor RB, Williams NS. Identification of NF-kappa B-regulated genes induced by TNFalpha utilizing expression profiling and RNA interference. Oncogene 2003;22:2054–64.
[138] Tian B, Brasier AR. Identification of a nuclear factor kappa B-dependent gene network. Recent Prog Horm Res 2003;58:95–130.
[139] Ricciardi-Castagnoli P, Granucci F. Opinion: interpretation of the complexity of innate immune responses by functional genomics. Nat Rev Immunol 2002;2:881–9.
[140] Baldwin Jr AS. Series introduction: the transcription factor NF-kappaB and human disease. J Clin Invest 2001;107:3–6.
[141] Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. J Clin Invest 2001;107:7–11.

[142] Silverman N, Maniatis T. NF-kappaB signaling pathways in mammalian and insect innate immunity. Genes Dev 2001;15:2321–42.
[143] Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999;18:6938–47.
[144] Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 2001;107: 241–6.
[145] Karin M, Lin A. NF-kappaB at the crossroads of life and death. Nat Immunol 2002;3:221–7.
[146] Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. Oncogene 2001;20:4188–97.
[147] Bhat-Nakshatri P, Sweeney CJ, Nakshatri H. Identification of signal transduction pathways involved in constitutive NF-kappaB activation in breast cancer cells. Oncogene 2002;21:2066–78.
[148] Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med 2001;194:1861–74.
[149] Kucharczak J, Simmons MJ, Fan Y, Gelinas C. To be, or not to be: NF-kappaB is the answer—role of Rel/NF-kappaB in the regulation of apoptosis. Oncogene 2003;22:8961–82.
[150] Garg AK, Hortobagyi GN, Aggarwal BB, Sahin AA, Buchholz TA. Nuclear factor-kappaB as a predictor of treatment response in breast cancer. Curr Opin Oncol 2003;15:405–11.
[151] Karin M, Yamamoto Y, Wang QM. The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 2004;3:17–26.
[152] Zambrowicz BP, Sands AT. Knockouts model the 100 best-selling drugs—will they model the next 100? Nat Rev Drug Discov 2003;2:38–51.
[153] Chen F, Demers LM, Shi X. Upstream signal transduction of NF-kappaB activation. Curr Drug Targets Inflamm Allergy 2002;1:137–49.
[154] Haefner B. NF-kappa B: arresting a major culprit in cancer. Drug Discov Today 2002;7:653–63.
[155] Heinrich M. Ethnobotany and natural products: the search for new molecules, new treatments of old diseases or a better understanding of indigenous cultures? Curr Top Med Chem 2003;3:141–54.
[156] O'Neill L. Inhibiting NF-kB. Trends Immunol 2001;22:478.
[157] Vanden Berghe W, De Bosscher K, Vermeulen L, De Wilde G, Haegeman G. Induction and repression of NF-kB-driven inflammatory genes. Ernst Schering Res Found Workshop 2002;40:233–78.
[158] Saccani S, Pantano S, Natoli G. Two waves of nuclear factor kappaB recruitment to target promoters. J Exp Med 2001;193:1351–9.
[159] Saccani S, Pantano S, Natoli G. p38-Dependent marking of inflammatory genes for increased NF-kappa B recruitment. Nat Immunol 2002;3:69–75.
[160] Saccani S, Natoli G. Dynamic changes in histone H3 Lys 9 methylation occurring at tightly regulated inducible inflammatory genes. Genes Dev 2002;16:2219–24.
[161] Smale ST, Fisher AG. Chromatin structure and gene regulation in the immune system. Annu Rev Immunol 2002;20:427–62.
[162] Holloway AF, Rao S, Chen X, Shannon MF. Changes in chromatin accessibility across the GM-CSF promoter upon T cell activation are dependent on nuclear factor kappaB proteins. J Exp Med 2003;197:413–23.
[163] Michael LF, Asahara H, Shulman AI, Kraus WL, Montminy M. The phosphorylation status of a cyclic AMP-responsive activator is modulated via a chromatin-dependent mechanism. Mol Cell Biol 2000;20:1596–603.
[164] Goriely S, Demonte D, Nizet S, De Wit D, Willems F, Goldman M, et al. Human IL-12(p35) gene activation involves selective remodeling of a single nucleosome within a region of the promoter containing critical Sp1-binding sites. Blood 2003;101:4894–902.
[165] Vermeulen L, De Wilde G, Damme PV, Vanden Berghe W, Haegeman G. Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). EMBO J 2003;22:1313–24.
[166] Schmitz ML, Bacher S, Kracht M. I kappa B-independent control of NF-kappa B activity by modulatory phosphorylations. Trends Biochem Sci 2001;26:186–90.
[167] Schreiber SL, Bernstein BE. Signaling network model of chromatin. Cell 2002;111:771–8.
[168] Holmberg CI, Tran SE, Eriksson JE, Sistonen L. Multisite phosphorylation provides sophisticated regulation of transcription factors. Trends Biochem Sci 2002;27:619–27.
[169] D'Alessandro T, Prasain J, Benton MR, Botting N, Moore R, Darley-Usmar V, et al. Polyphenols, inflammatory response, and cancer prevention: chlorination of isoflavones by human neutrophils. J Nutr 2003;133:3773S–7S.
[170] Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, et al. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat Res 2001;480–481:243–68.
[171] Regal JF, Fraser DG, Weeks CE, Greenberg NA. Dietary phytoestrogens have anti-inflammatory activity in a guinea pig model of asthma. Proc Soc Exp Biol Med 2000;223:372–8.
[172] Verdrengh M, Jonsson IM, Holmdahl R, Tarkowski A. Genistein as an anti-inflammatory agent. Inflamm Res 2003;52:341–6.
[173] Kang JL, Lee HW, Lee HS, Pack IS, Chong Y, Castranova V, et al. Genistein prevents nuclear factor-kappa B activation and acute lung injury induced by lipopolysaccharide. Am J Respir Crit Care Med 2001;164:2206–12.
[174] Baxa DM, Yoshimura FK. Genistein reduces NF-kappa B in T lymphoma cells via a caspase-mediated cleavage of I kappa B alpha. Biochem Pharmacol 2003;66:1009–18.
[175] Dixon-Shanies D, Shaikh N. Growth inhibition of human breast cancer cells by herbs and phytoestrogens. Oncol Rep 1999;6:1383–7.
[176] Davis JN, Kucuk O, Sarkar FH. Genistein inhibits NF-kappa B activation in prostate cancer cells. Nutr Cancer 1999;35:167–74.
[177] Li Y, Ellis KL, Ali S, El-Rayes BF, Nedeljkovic-Kurepa A, Kucuk O, et al. Apoptosis-inducing effect of chemotherapeutic agents is potentiated by soy isoflavone genistein, a natural inhibitor of NF-kappaB in BxPC-3 pancreatic cancer cell line. Pancreas 2004;28:e90–5.
[178] Buchler P, Gukovskaya AS, Mouria M, Buchler MC, Buchler MW, Friess H, et al. Prevention of metastatic pancreatic cancer growth in vivo by induction of apoptosis with genistein, a naturally occurring isoflavonoid. Pancreas 2003;26:264–73.
[179] Maret A, Clamens S, Delrieu I, Elhage R, Arnal JF, Bayard F. Expression of the interleukin-6 gene is constitutive and not regulated by estrogen in rat vascular smooth muscle cells in culture. Endocrinology 1999;140:2876–82.
[180] Harrington WR, Sheng S, Barnett DH, Petz LN, Katzenellenbogen JA, Katzenellenbogen BS. Activities of estrogen receptor alpha- and beta-selective ligands at diverse estrogen responsive gene sites mediating transactivation or transrepression. Mol Cell Endocrinol 2003;206:13–22.
[181] Cerillo G, Rees A, Manchanda N, Reilly C, Brogan I, White A, et al. The oestrogen receptor regulates NFkappaB and AP-1 activity in a cell-specific manner. J Steroid Biochem Mol Biol 1998;67:79–88.
[182] Jenkins DJ, Kendall CW, Connelly PW, Jackson CJ, Parker T, Faulkner D, et al. Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. Metabolism 2002;51:919–24.
[183] Stein B, Yang MX. Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-kappa B and C/EBP beta. Mol Cell Biol 1995;15:4971–9.
[184] Ray P, Ghosh SK, Zhang DH, Ray A. Repression of interleukin-6 gene expression by 17 beta-estradiol: inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF-kappa B by the estrogen receptor. FEBS Lett 1997;409:79–85.

[185] Galien R, Garcia T. Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-kappaB site. Nucl Acids Res 1997;25:2424–9.
[186] Sun WH, Keller ET, Stebler BS, Ershler WB. Estrogen inhibits phorbol ester-induced I kappa B alpha transcription and protein degradation. Biochem Biophys Res Commun 1998;244:691–5.
[187] Harnish DC, Scicchitano MS, Adelman SJ, Lyttle CR, Karathanasis SK. The role of CBP in estrogen receptor cross-talk with nuclear factor-kappaB in HepG2 cells. Endocrinology 2000;141:3403–11.
[188] Kurebayashi S, Miyashita Y, Hirose T, Kasayama S, Akira S, Kishimoto T. Characterization of mechanisms of interleukin-6 gene repression by estrogen receptor. J Steroid Biochem Mol Biol 1997;60:11–7.
[189] Evans MJ, Eckert A, Lai K, Adelman SJ, Harnish DC. Reciprocal antagonism between estrogen receptor and NF-kappaB activity in vivo. Circ Res 2001;89:823–30.
[190] Evans MJ, Lai K, Shaw LJ, Harnish DC, Chadwick CC. Estrogen receptor alpha inhibits IL-1beta induction of gene expression in the mouse liver. Endocrinology 2002;143:2559–70.
[191] Delerive P, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. J Biol Chem 1999;274:32048–54.
[192] Ruan H, Pownall HJ, Lodish HF. Troglitazone antagonizes tumor necrosis factor-alpha-induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NF-kappaB. J Biol Chem 2003;278:28181–92.
[193] Wissink S, van Heerde EC, vand der Burg B, van der Saag PT. A dual mechanism mediates repression of NF-kappaB activity by glucocorticoids. Mol Endocrinol 1998;12:355–63.
[194] De Bosscher K, Vanden Berghe W, Haegeman G. Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: negative interference of activated glucocorticoid receptor with transcription factors. J Neuroimmunol 2000;109:16–22.
[195] Keller ET, Chang C, Ershler WB. Inhibition of NFkappaB activity through maintenance of IkappaBalpha levels contributes to dihydrotestosterone-mediated repression of the interleukin-6 promoter. J Biol Chem 1996;271:26267–75.
[196] Palvimo JJ, Reinikainen P, Ikonen T, Kallio PJ, Moilanen A, Janne OA. Mutual transcriptional interference between RelA and androgen receptor. J Biol Chem 1996;271:24151–6.
[197] Kalkhoven E, Wissink S, van der Saag PT, van der Burg B. Negative interaction between the RelA(p65) subunit of NF-kappaB and the progesterone receptor. J Biol Chem 1996;271:6217–24.
[198] McKay LI, Cidlowski JA. Cross-talk between nuclear factor-kappa B and the steroid hormone receptors: mechanisms of mutual antagonism. Mol Endocrinol 1998;12:45–56.
[199] Tian Y, Ke S, Denison MS, Rabson AB, Gallo MA. Ah receptor and NF-kappaB interactions, a potential mechanism for dioxin toxicity. J Biol Chem 1999;274:510–5.
[200] Castrillo A, Joseph SB, Marathe C, Mangelsdorf DJ, Tontonoz P. Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. J Biol Chem 2003;278:10443–9.
[201] Harant H, Andrew PJ, Reddy GS, Foglar E, Lindley IJ. 1alpha,25-Dihydroxyvitamin D3 and a variety of its natural metabolites transcriptionally repress nuclear-factor-kappaB-mediated interleukin-8 gene expression. Eur J Biochem 1997;250:63–71.
[202] D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3: involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. J Clin Invest 1998;101:252–62.
[203] Frasor J, Stossi F, Danes JM, Komm B, Lyttle CR, Katzenellenbogen BS. Selective estrogen receptor modulators: discrimination of agonistic versus antagonistic activities by gene expression profiling in breast cancer cells. Cancer Res 2004;64:1522–33.
[204] Dang ZC, Audinot V, Papapoulos SE, Boutin JA, Lowik CW. Peroxisome proliferator-activated receptor gamma (PPARgamma) as a molecular target for the soy phytoestrogen genistein. J Biol Chem 2003;278:962–7.
[205] Amakura Y, Tsutsumi T, Nakamura M, Kitagawa H, Fujino J, Sasaki K, et al. Activation of the aryl hydrocarbon receptor by some vegetable constituents determined using in vitro reporter gene assay. Biol Pharm Bull 2003;26:532–9.
[206] Suetsugi M, Su L, Karlsberg K, Yuan YC, Chen S. Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors. Mol Cancer Res 2003;1:981–91.
[207] Driggers PH, Segars JH. Estrogen action and cytoplasmic signaling pathways. Part II. The role of growth factors and phosphorylation in estrogen signaling. Trends Endocrinol Metab 2002;13:422–7.
[208] Schiff R, Massarweh S, Shou J, Osborne CK. Breast cancer endocrine resistance: how growth factor signaling and estrogen receptor coregulators modulate response. Clin Cancer Res 2003;9:447S–54S.
[209] Bennasroune A, Gardin A, Aunis D, Cremel G, Hubert P. Tyrosine kinase receptors as attractive targets of cancer therapy. Crit Rev Oncol Hematol 2004;50:23–38.
[210] Ali S, Coombes C. Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev Mol Cell Biol 2002;2:101–15.
[211] Bunone G, Briand PA, Miksicek RJ, Picard D. Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. EMBO J 1996;15:2174–83.
[212] Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, et al. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 1995;270:1491–4.
[213] Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. J Biol Chem 2001;276:9817–24.
[214] Bhat-Nakshatri P, Campbell RA, Patel NM, Newton TR, King AJ, Marshall MS, et al. Tumour necrosis factor and PI3-kinase control oestrogen receptor alpha protein level and its transrepression function. Br J Cancer 2004;90:853–9.
[215] Vermeulen L, De Wilde G, Notebaert S, Vanden Berghe W, Haegeman G. Regulation of the transcriptional activity of the nuclear factor-kappaB p65 subunit. Biochem Pharmacol 2002;64:963–70.
[216] Segars JH, Driggers PH. Estrogen action and cytoplasmic signaling cascades. Part I. Membrane-associated signaling complexes. Trends Endocrinol Metab 2002;13:349–54.
[217] Lu T, Sathe SS, Swiatkowski SM, Hampole CV, Stark GR. Secretion of cytokines and growth factors as a general cause of constitutive NFkappaB activation in cancer. Oncogene 2004;23:2138–45.
[218] Maggiolini M, Vivacqua A, Fasanella G, Recchia AG, Sisci D, Pezzi V, et al. The G protein-coupled receptor GPR30 mediates c-fos up-regulation by 17beta-estradiol and phytoestrogens in breast cancer cells. J Biol Chem 2004;279:27008–16.
[219] Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. J Biol Chem 2001;276: 36869–72.
[220] Farach-Carson MC, Davis PJ. Steroid hormone interactions with target cells: cross talk between membrane and nuclear pathways. J Pharmacol Exp Ther 2003;307:839–45.
[221] Losel R, Wehling M. Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol 2003;4:46–56.
[222] Collins P, Webb C. Estrogen hits the surface. Nat Med 1999;5: 1130–1.
[223] Filardo EJ, Quinn JA, Bland KI, Frackelton Jr AR. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. Mol Endocrinol 2000;14:1649–60.
[224] Filardo EJ. Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel

signaling pathway with potential significance for breast cancer. J Steroid Biochem Mol Biol 2002;80:231–8.
[225] Filardo EJ, Quinn JA, Frackelton Jr AR, Bland KI. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. Mol Endocrinol 2002; 16:70–84.
[226] Tachibana H, Koga K, Fujimura Y, Yamada K. A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol 2004;11:380–1.
[227] Larner AC, Finbloom DS. Protein tyrosine phosphorylation as a mechanism which regulates cytokine activation of early response genes. Biochim Biophys Acta 1995;1266:278–87.
[228] Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000;103:211–25.
[229] Darnay BG, Aggarwal BB. Inhibition of protein tyrosine phosphatases causes phosphorylation of tyrosine-331 in the p60 TNF receptor and inactivates the receptor-associated kinase. FEBS Lett 1997;410:361–7.
[230] Huang WC, Chen JJ, Inoue H, Chen CC. Tyrosine phosphorylation of I-kappa B kinase alpha/beta by protein kinase C-dependent c-Src activation is involved in TNF-alpha-induced cyclooxygenase-2 expression. J Immunol 2003;170:4767–75.
[231] Huang WC, Chen JJ, Chen CC. c-Src-dependent tyrosine phosphorylation of IKKbeta is involved in tumor necrosis factor-alpha-induced intercellular adhesion molecule-1 expression. J Biol Chem 2003;278:9944–52.
[232] Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 2003;3:745–56.
[233] Chen LY, Zuraw BL, Zhao M, Liu FT, Huang S, Pan ZK. Involvement of protein tyrosine kinase in toll-like receptor 4-mediated NF-kappa B activation in human peripheral blood monocytes. Am J Physiol Lung Cell Mol Physiol 2003;284:L607–13.
[234] Hirota K, Murata M, Itoh T, Yodoi J, Fukuda K. Redox-sensitive transactivation of epidermal growth factor receptor by tumor necrosis factor confers the NF-kappa B activation. J Biol Chem 2001;276:25953–8.
[235] Hou MF, Lin SB, Yuan SS, Tsai SM, Wu SH, Ou-Yang F, et al. The clinical significance between activation of nuclear factor kappa B transcription factor and overexpression of HER-2/neu oncoprotein in Taiwanese patients with breast cancer. Clin Chim Acta 2003;334:137–44.
[236] Zwick E, Bange J, Ullrich A. Receptor tyrosine kinase signalling as a target for cancer intervention strategies. Endocrinol Relat Cancer 2001;8:161–73.
[237] Burgess AW, Cho HS, Eigenbrot C, Ferguson KM, Garrett TP, Leahy DJ, et al. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. Mol Cell 2003;12:541–52.
[238] Lee AV, Schiff R, Cui X, Sachdev D, Yee D, Gilmore AP, et al. New mechanisms of signal transduction inhibitor action: receptor tyrosine kinase down-regulation and blockade of signal transactivation. Clin Cancer Res 2003;9:516S–23S.
[239] Morin MJ. From oncogene to drug: development of small molecule tyrosine kinase inhibitors as anti-tumor and anti-angiogenic agents. Oncogene 2000;19:6574–83.
[240] Yang SH, Sharrocks AD, Whitmarsh AJ. Transcriptional regulation by the MAP kinase signaling cascades. Gene 2003;320:3–21.
[241] Devin A, Lin Y, Liu ZG. The role of the death-domain kinase RIP in tumour-necrosis-factor-induced activation of mitogen-activated protein kinases. EMBO Rep 2003;4:623–7.
[242] Chang F, Steelman LS, Lee JT, Shelton JG, Navolanic PM, Blalock WL, et al. Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. Leukemia 2003;17:1263–93.
[243] Sebolt-Leopold JS. Development of anticancer drugs targeting the MAP kinase pathway. Oncogene 2000;19:6594–9.
[244] Schulze-Osthoff K, Beyaert R, Vandevoorde V, Haegeman G, Fiers W. Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO J 1993;12:3095–104.
[245] Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J 1991;10:2247–58.
[246] Mihm S, Ennen J, Pessara U, Kurth R, Droge W. Inhibition of HIV-1 replication and NF-kappa B activity by cysteine and cysteine derivatives. Aids 1991;5:497–503.
[247] Adler V, Yin Z, Tew KD, Ronai Z. Role of redox potential and reactive oxygen species in stress signaling. Oncogene 1999;18: 6104–11.
[248] Bowie A, O'Neill LA. Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries. Biochem Pharmacol 2000;59:13–23.
[249] Schreck R, Grassmann R, Fleckenstein B, Baeuerle PA. Antioxidants selectively suppress activation of NF-kappa B by human T-cell leukemia virus type I Tax protein. J Virol 1992;66:6288–93.
[250] Hayakawa M, Miyashita H, Sakamoto I, Kitagawa M, Tanaka H, Yasuda H, et al. Evidence that reactive oxygen species do not mediate NF-kappaB activation. EMBO J 2003;22:3356–66.
[251] Brash DE, Havre PA. New careers for antioxidants. Proc Natl Acad Sci USA 2002;99:13969–71.
[252] Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, et al. NF-kappaB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. EMBO J 2003;22:3898–909.
[253] Cotelle N. Role of flavonoids in oxidative stress. Curr Top Med Chem 2001;1:569–90.
[254] van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Bernards R, et al. Expression profiling predicts outcome in breast cancer. Breast Cancer Res 2003;5:57–8.
[255] van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–6.
[256] Kaput J, Rodriguez RL. Nutritional genomics: the next frontier in the postgenomic era. Physiol Genomics 2004;16:166–77.
[257] Ferrucci L, Guralnik JM. Inflammation, hormones, and body composition at a crossroad. Am J Med 2003;115:501–2.
[258] Bornstein SR, Rutkowski H. The adrenal hormone metabolism in the immune/inflammatory reaction. Endocrinol Res 2002;28:719–28.
[259] Path G, Scherbaum WA, Bornstein SR. The role of interleukin-6 in the human adrenal gland. Eur J Clin Invest 2000;30(Suppl 3):91–5.
[260] Cutolo M, Sulli A, Pizzorni C, Craviotto C, Straub RH. Hypothalamic–pituitary–adrenocortical and gonadal functions in rheumatoid arthritis. Ann NY Acad Sci 2003;992:107–17.
[261] Vanden Berghe W, Plaisance S, Boone E, De Bosscher K, Schmitz ML, Fiers W, et al. p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-kappaB p65 transactivation mediated by tumor necrosis factor. J Biol Chem 1998;273:3285–90.
[262] Vanden Berghe W, De Bosscher K, Boone E, Plaisance S, Haegeman G. The nuclear factor-kappaB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter. J Biol Chem 1999;274:32091–8.
[263] De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. Endocrinol Rev 2003;24:488–522.
[264] Vanden Berghe W, Vermeulen L, Delerive P, De Bosscher K, Staels B, Haegeman G. A paradigm for gene regulation: inflammation, NF-kappaB and PPAR. Adv Exp Med Biol 2003;544:181–96.
[265] Trubiani O, Di Giulio C, Tripodi D, Bianchi G, Paganelli R, Di Primio R. Thymic sensitivity to hypoxic condition in young and old rats: age-dependent expression of NF-kappaB. Exp Gerontol 2002;37:1077–88.

[266] Bernard D, Gosselin K, Monte D, Vercamer C, Bouali F, Pourtier A, et al. Involvement of Rel/nuclear factor-kappaB transcription factors in keratinocyte senescence. Cancer Res 2004;64:472–81.
[267] Bernard D, Mont D, Vandenbunder B, Abbadie C. Involvement of rel/nf-B transcription factors in cellular senescence. Sci World J 2001;1:67.
[268] Dennis C. Epigenetics and disease: altered states. Nature 2003;421: 686–8.
[269] Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33(Suppl):245–54.
[270] Calemine J, Zalenka J, Karpuzoglu-Sahin E, Ward DL, Lengi A, Ahmed SA. The immune system of geriatric mice is modulated by estrogenic endocrine disruptors (diethylstilbestrol, alpha-zearalanol, and genistein): effects on interferon-gamma. Toxicology 2003;194: 115–28.
[271] Islander U, Erlandsson MC, Hasseus B, Jonsson CA, Ohlsson C, Gustafsson JA, et al. Influence of oestrogen receptor alpha and beta on the immune system in aged female mice. Immunology 2003; 110:149–57.
[272] Giacconi R, Cipriano C, Muzzioli M, Gasparini N, Orlando F, Mocchegiani E. Interrelationships among brain, endocrine and immune response in ageing and successful ageing: role of metallothionein III isoform. Mech Ageing Dev 2003;124:371–8.
[273] Barud W, Palusinski R, Beltowski J, Wojcicka G. Inverse relationship between total testosterone and anti-oxidized low density lipoprotein antibody levels in ageing males. Atherosclerosis 2002;164: 283–8.
[274] Mocchegiani E, Giacconi R, Cipriano C, Muzzioli M, Gasparini N, Moresi R, et al. MtmRNA gene expression, via IL-6 and glucocorticoids, as potential genetic marker of immunosenescence: lessons from very old mice and humans. Exp Gerontol 2002;37:349–57.
[275] Brown-Borg HM. Hormonal regulation of aging and life span. Trends Endocrinol Metab 2003;14:151–3.
[276] Issa JP. Age-related epigenetic changes and the immune system. Clin Immunol 2003;109:103–8.
[277] Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann NY Acad Sci 2000;908:244–54.
[278] Prior RL. Fruits and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr 2003;78:570S–8S.
[279] Hu HL, Forsey RJ, Blades TJ, Barratt ME, Parmar P, Powell JR. Antioxidants may contribute in the fight against ageing: an in vitro model. Mech Ageing Dev 2000;121:217–30.