Review

Cancer protection of soy resembles cancer protection during pregnancy

Uwe D. Rohr1*, Anca G. Gocan1, Doris Bachg2 and Adolf E. Schindler3

1 The Vienna Stress Relief Clinic, Georgia, USA
2 Biofocus, Institute for Molecular Oncology, Recklinghausen, Germany
3 Institute for Medical Research and Education, Essen, Germany

Abstract

It has been established that carrying a pregnancy to full-term at an early age can protect against contracting cancer by up to 50% in later life. The trophoblast theory of cancer states that trophoblast and cancer tissue are very similar. New findings suggest that the loss of fetal cells during pregnancy resemble those cells responsible for causing metastasis in cancer. Fetal cells and spreading cancer cells are highly proliferative. They are similar to stem cells, exhibiting no or low hormone receptor expression, and require a hormone receptor independent mechanism for control. Control of membrane stability during pregnancy is of vital importance for a successful pregnancy and is mediated by androstenediol and 2-methoxyestradiol. 2-Methoxyestradiol has no hormone receptor affinity and elicits strong anticancer effects particularly against cancer stem cells and fetal cells, for which currently no treatment has yet been established. There is a discussion whether pregnancy reduces cancer stem cells in the breast. Soy isoflavones are structurally similar to both hormones, and elicit strong anticancer effects and antiangiogenesis via inhibition of NF-κB, even in hormone receptor independent breast cancers seen in epidemiologic studies. The trophoblast theory of cancer could help to explain why soy baby nutrition formulas have no effect on baby physiology, other than the nutritional aspect, although soy elicits many effects on the adult immune system. To survive the immune system of the mother, the immune system of the fetus has to be separated; otherwise, the reduction of the immune system in the mother, a necessary feature for the blastocyst to grow, would immediately reduce the immunity for the fetus and endanger its survival. Similar to a fetus, newly born babies show immune insensitive to Th1 and Th2 cytokines, which are necessary and crucial for regulating the immune system of the mother, thus raising the risk of the baby of developing allergies and neurodermatitis. Gene expression studies in vitro as well as in circulating tumor cells from patients consuming a fermented soy product support the antiangiogenic as well as antiproliferative effects of soy.

Keywords: androstenediol; breast cancer; cachexia; cancer; capillary; curcumin; cytokines; depression; fermented soy; fetus; isoflavones; 2-methoxyestradiol; soy; soy nutritional formula; Th1; Th2; trophoblast.

Introduction

Whereas plant-based formulations have been used to treat cancer for centuries, current treatments usually involve poisonous mustard gas, chemotherapy, radiation, and targeted therapies (1). Natural products have been the most significant source of drugs (2). Their dominant role in cancer chemotherapeutics is clear with approximately 74% of anticancer compounds being either natural products or natural product-derived, mostly via inhibition of mitosis (2–4). Numerous bioactive chemical compounds of plant origin influence not only mitosis but they are also known to increase apoptosis via modifying the nuclear factor kappa beta (NF-κB) transcription factor and angiogenesis, and can also affect blood vessels in tumors and reduce cancer growth (Figures 1 and 2) (3, 4).

NF-κB has a key role in many physiological processes such as innate and adaptive immune response, cell proliferation, cell death, and inflammation (5). It has become clear that aberrant regulation of NF-κB and the signaling pathways that control its activity are involved in cancer development and progression and also appear resistant to chemotherapy and radiotherapy (6). Although the dominant role NF-κB plays has been recognized, its therapeutic use in cancer treatment has not yet been fully established (5, 6).

The first generation approach to antiangiogenesis beyond the NF-κB mechanism via anti-vascular endothelial growth factor (anti-VEGF) inhibition was aimed to “destroy and suppress” blood vessels in cancers (7, 8). This concept was less effective in clinical trials than anticipated (7, 8). Newer evaluations reveal that capillaries in cancer have lost their stability and are leaky and unstable so that the tumor can spread and grow (7, 8).

Soy ingredients show strong effects on NF-κB (Figures 1 and 2), and it was shown in several epidemiological meta-analyses that consumption of soy food, particularly soy isoflavones, protects against various types of cancers, particularly against breast cancer (9, 10). The majority of animal studies and in vitro cell culture studies support these
Figure 1  Structural comparison of natural hormones with plant-derived substances. Three anticancer mechanisms are possible: antiangiogenesis, antimitosis, and antiproliferation.

Figure 2  Schematic depiction of the relationship between cancer and inflammation. Both are related via the MAP-kinase/NF-kB cascade. Silencing via estrogen receptor beta (ER-β) by soy isoflavones results in decreased cytokines such as interleukin 6 (IL-6) and increased apoptosis, resulting in reduced inflammation and cancer. The increase of IL-6 results in increased cachexia (appetite loss) and increased cancer risk.

observations (10–12). However, a few animal experiments produced contradictory results and do not support a cancer protection hypothesis (13).

Although many investigations have been conducted, it is still not clear how soy or its ingredient soy isoflavones achieve anticancer activity. A recent very important epidemiologic finding claims that soy protection against cancer is independent from the estrogen receptor (ER) status (Figure 3), and irrespective of whether a breast cancer is ER-positive (ER+) or ER-negative (ER-) or whether patients are Oriental (14–16) or Caucasian women (17). This finding clarifies earlier epidemiologic studies, which were not specifically designed to investigate this question. Authors of these new epidemiological findings seek a new explanation concerning soy cancer protection mechanisms beyond hormone receptors (16, 17).

The finding of soy protection against ER—breast cancer is of tremendous clinical importance, because women with ER—breast cancer have a poor prognosis, suffer from more aggressive tumors, and currently no medical prevention has been established, leaving those women with the highest risk and currently without any medical protection (18).

A new model describing cancer origin, recurrence, and progression suggests that cancer evolves from a small subfraction of cells, which are stem cell-like, less differentiated, and have no or only few hormone receptors (Figures 4–6) (20, 21). Whereas differentiated cells show hormone receptor expression in all cells, cancer stem cells show low hormone receptor expression (Figure 6) (19). It was shown in a clinical study in Caucasian women diagnosed with breast cancer that the primary tumor exhibits 68% ER + cancer cells, whereas in the blood circulating tumor cells show only 35% ER + expression in circulating cancer cells (Figures 4 and 5) (22). This clearly supports the assumption that current adjuvant therapy is restricted to ER+ breast cancer, because it is effective via blocking the classical ER, what is now coined as ER-α (23).

Cancer stem cells fail to respond to radiation or chemotherapy (Figure 4) (24–26). They leave the primary tumor and cause metastases (Figure 5) (24–26). Cancer stem cells have no or low hormone receptor expression. However, they do express NF-κB (6, 27) and could very well be silenced via inhibition of NF-κB. The finding that soy isoflavones silence ER—breast cancer, which is more stem cell-like and has no or low ER expression, raises the question of its mechanism of action. NF-κB can be altered by soy isoflavones; it can therefore influence cancer stem cells via this mechanism as discussed in this review.
It appears that cancer shares many properties with a trophoblast (Figure 7) (28, 29). After implantation of a fertilized egg in the uterus, the immunity of the mother has to be carefully balanced with the immunity of the trophoblast so that the immune system of the mother does not reject the trophoblast. As will be shown, regulating the immunity of the mother during pregnancy is correlated to hormones structurally similar to soy isoflavones, such as androstenediol and 2-methoxyestradiol, all of which are increased during pregnancy (Figure 8) (30, 31). They are not only structurally similar (Figure 1) but they also exhibit strong antiproliferative effects and have strong antiangiogenic effects (30–34). Additionally, 2-methoxyestradiol, in contrast to its misleading name, has no affinity to any hormone receptor and elicits its antiproliferative effect via non-hormonal receptors in stem cells, which are more aggressive than differentiated cancer cells (Figure 9) (19). The increase during pregnancy of these hormones might be necessary to reduce highly active fetal cells, which leave the uterus and nest in precancerous tissues of the mother, which could facilitate angiogenesis and cancer growth, because fetal cells are highly proliferative (35).

Full-term pregnancies are associated with long-term reductions in maternal risk of breast cancer, but the biological determinants of the protection are unknown and many factors could contribute (22). Nevertheless, immunity modification by steroids produced by the mother during pregnancy share many similarities with soy isoflavones. Clinical trials and animal studies have shown that maternal steroids enhance immunity and are strong anticancer agents against breast cancer (31–34).

The steroidal hormones mediate their immune modifying effects via Th1 cytokines, which can in return alter membrane permeability (30). In particular, androstenediol has a very strong protective immune modifying effect via...
cytokines (Figures 10–13) (37). A clinical trial with breast cancer patients revealed a reduction of Th1 cytokines and a reduction of symptoms related to Th1 cytokines after consuming fermented soy (Figure 14) (38, 39). It is now recognized that cytokines in the blood of cancer patients are released from immune cells within the tumor, increasing the surrounding capillary membrane permeability, leading to the spread of tumor cells and cytokines, and causing metastasis and discomfort (Figure 15) (40–46). The Th1 cytokines in the blood are released from the tumor causing leaks in the blood capillaries or trophoblast membrane. This situation is similar to complications in pregnancy and can result in possible miscarriage, premature birth, or other complications during labor or birth (Figures 11 and 12).

Although androstenediol and 2-methoxyestradiol elicit strong immunological effects in the mother and are present in the amniotic fluid, they have no effect on the fetus (31). The necessary separation of the immunity of the mother and the trophoblast/fetus could help to explain why soy baby formulas have no effect on the immunity of the baby. The reduced immunity in babies could well be a remnant of fetal development. As the baby progresses through childhood, a stronger immunity system begins to be established. Thus, studies suggest that consumption of soy during early childhood begins to have an effect, protecting against cancer later in life.

Present studies suggest that it could be advantageous to include agents modifying NF-κB during cancer therapy, because they do not interfere with mitosis on the genetic molecular level (Figure 16) and could protect the patient against the side effects of chemotherapy.

**Breast cancer stem cell model**

Breast cancer remains a significant public health problem despite advances in our understanding of the molecular and cellular events that underpin the disease (25, 26, 48). Recently, a new model has been proposed. This model links breast cancer cells to stem cells and progenitors, an observation originally made in other cancer entities (25). It hypothesizes that the tumors originated from a small population of undifferentiated cells, which have no or only small amounts of hormone receptors (49). These cells can undergo self-renewal and are able to generate a large number of partially differentiated cells, which constitute the bulk of the tumor.

These cancer stem cells resemble adult stem and progenitor cells found in the normal breast, but are deregulated from their patterns of proliferation and differentiation (19). They could originate from normal stem cells or from more differentiated progenitors and lose their normal growth restraints through a series of oncogenic mutations that deregulate a small number of central signaling pathways (48, 49). It was found that ER was expressed in circulating tumor cells (CTCs) up to 35%, whereas ER expression was found in...
Figure 8  Schematic of the relationship between androstenediol, 2-methoxyestradiol, and Th1 cytokines. The risk of losing a baby in the first trimester is caused by the increase of Th1 cytokines. The increase is necessary to reduce the immunity of the mother so that the trophoblast can be fed with nutrients and O2. The increase of Th1 cytokines can cause vomiting, fatigue, and discomfort of the mother, which is normally reduced if the pregnancy is continued. See Ref. (15).

The risk of losing a baby in the first trimester is caused by the increase of Th1 cytokines. The increase is necessary to reduce the immunity of the mother so that the trophoblast can be fed with nutrients and O2. The increase of Th1 cytokines can cause vomiting, fatigue, and discomfort of the mother, which is normally reduced if the pregnancy is continued. See Ref. (15).

approximately 68% of all cells at the primary site (Figures 4 and 5) (50). This reduces the possibility to limit cell growth of CTCs through aromatase inhibitors or tamoxifen, because they reduce only cancer cells, which express ER (23).

Despite the fact that hormone receptors could have limited possibilities to regulate stem cells and cancer stem cells, they do respond to changes in NF-κB (6, 51, 52).

Breast cancer stem cells do not respond to chemotherapy, radiotherapy, or adjuvant therapy. Also, it is estimated that at the time of diagnosis cancer stem cells have left the primary tumor in 40% of all cases and are distributed in the body via blood, bone, or lymphatic system. Therefore, targeted treatment of disseminated cancer stem cells is of vital importance, because surgical removal of the primary tumor will not reduce distributed tumor stem cells (25, 26, 48, 49, 51).

Full-term pregnancy at young age causes cancer protection

By completing a pregnancy at a young age, a woman reduces the risk of breast cancer by up to 50% over her lifetime (53, 54). A similar protective effect has been observed in rodent models with an early pregnancy by using chemical carcinogens to induce cancer (54). However, the mechanisms responsible for this protective effect remain unclear.

Cancer is predominately a disease of old age; however, every 20th child is born with leukemic cancer cells, although the child might not necessarily develop the disease (55). Therefore, the threat of cancer is not only a threat to the mother but also to the child.

Despite the fact that pregnancy protects against many cancers, such as breast cancer, it is now more and more recognized that pregnancy also generates a pre-cancerous environment (56, 57). The Italian physician Bernardino Ramazzini was the first to describe how catholic nuns suffer from high rates of breast cancer (56, 57). Contradicting observations illustrated how some cancers, such as neurofibroma or melanoma, grow rapidly in young mothers during pregnancy (19, 35).

Many similarities between cancer and trophoblasts continue to be observed (28, 29). Cancer tissue manipulates its own environment to receive nutrients, oxygen, and alters barrier functions so it can create space to grow.

Pregnancy increases breast tissue differentiation, a feature that protects against cancer. New investigations illustrate how two hormones, androstenediol (30) and 2-methoxyestradiol (19), are elevated during pregnancy and show strong antiproliferative (Figure 9) and antiangiogenic effects, stabilizing capillary membranes and at the same time act in an antiproliferative manner, particularly against cancer stem cells believed to cause and spread cancer (28, 48).

Plant substances with antiangiogenic and antiproliferative properties

Plant growth is silenced in times of environmental stress, e.g., in winter or in dry season (58, 59). Plant derived compounds show strong antiproliferative effects and antiangiogenesis (Figures 1 and 17). Because most cancers are caused by deregulation of as many as 500 different genes, agents that target multiple gene products are needed for prevention and treatment of cancer beyond mitosis (60). Curcumin, e.g., is a diferuloylmethane derived from the Indian spice turmeric (popularly called ‘‘curry powder’’) that has been shown to interfere with multiple cell signaling pathways, including cell cycle (cyclin D1 and cyclin E), apoptosis (activation of caspases and downregulation of antiapoptotic gene products), proliferation (HER-2, EGFR, and AP-1), survival (PI3K/
AKT pathway), invasion (MMP-9 and adhesion molecules), angiogenesis (VEGF), metastasis (CXCR-4) and inflammation (NF-κB, TNF-α, IL-6, IL-1, COX-2, and 5-LOX), and activate the antiproliferative ER-β (61–63). It is reported that the activity of curcumin against leukemia and lymphoma, gastrointestinal cancers, genitourinary cancers, breast cancer, ovarian cancer, head and neck squamous cell carcinoma, lung cancer, melanoma, neurological cancers, and sarcoma reflects its ability to affect multiple targets (61, 62).

Many molecular anticancer effects of soy isoflavones are similar to curcumin, particularly antiangiogenesis and anti-proliferation, via inhibiting NF-κB. Out of the approximately 10,000 published isoflavone studies in peer-reviewed publications, almost one-fifth pertain to its antitumor capabilities and describe its mechanism of action in normal and malignant cells, animal models, in vitro experiments, or phase I/II clinical trials (64). The soy isoflavone genistein and daidzein, which are abundant in plants, particularly in soybeans, reduce chronic inflammation and cancer in Asian (65–68) as well as in Western populations (69–71). Migration studies have shown that after successive generations, breast cancer incidence in Asian women becomes similar to that of Western women (47). Furthermore, an increasing incidence of breast cancer among Japanese women parallels the Westernization of the Japanese diet (72). These new findings contradict the assumption that soy can protect only Asian women against breast cancer due to a genetic susceptibility. Only recent epidemiological studies show that soy isoflavones are effective in first-line as well as in second-line prevention of breast cancer (17, 47). A very important finding is that soy is equally protective against ER+ and ER– breast cancer (Figure 3). The finding that soy isoflavones protect against ER– tumors supports the assumption that they are similar to 2-methoxyestradiol to reduce cancer cell growth in stem cell-like cancers that have no or reduced hormone receptors and are more proliferative and are more aggressive than ER+ tumors.
**Trophoblast: theory of cancer**

It was the British developmental biologist, John Beard (1858–1924), who first to suggest that to understand anticanic treatment, the similarity between tumors and trophoblast needs to be discussed (28, 29). Trophoblasts provide protection by completely surrounding the embryo, while carrying nutrients and oxygen from the blood of the mother to that of the developing fetus. The word trophoblast means ‘original feeding tissue’ and was named after the Dutch embryologist Ambrosius Hubrecht (1853–1915) (28, 29). In the early part of the 20th century, scientists began to notice a remarkable similarity between trophoblastic cells and cancer. It was said, that if you mixed up microscopic slides of both trophoblasts and cancer, you could not tell the difference (28, 29). Both tumor tissue and trophoblast are highly proliferative, migratory, and invasive with an almost limitless ability to perpetuate.

The main difference between cancer and trophoblasts is that growth of trophoblasts is a natural self-contained process, limited to the environment of the uterus. In rare cases, however, trophoblasts can escape and can lead to chorioangioma carcinoma, a highly malignant form of cancer (Figure 7) (28, 29).

The cancer-like growth of a trophoblast is kept under control by a cascade of hormonal and cytokine signals (36). New investigations show that this process is actively supported in the first trimester of pregnancy by a decrease in androstenediol and increases in Th1 cytokine (e.g., TNF-α, IL-6) (Figures 8, 10, 11) (30). Generally, in pregnancy a direct correlation exists between androstenediol and Th1 cytokines (Figure 8) (30).

A reduction of the DHEA metabolite androstenediol in the first trimester reduces immunity in pregnancy, so that the fragile fetus is not rejected by the immune system of the mother (Figure 8) (30). It is interesting to note that fatigue and discomfort in the beginning of the pregnancy could be early signs of trophoblast proliferation and could indicate a possible risk of cancer. It was said, that if you mixed up microscopic slides of both trophoblasts and cancer, you could not tell the difference (28, 29). Both tumor tissue and trophoblast are highly proliferative, migratory, and invasive with an almost limitless ability to perpetuate.

This adaptation process, reduction of immunity in the first trimester, is of vital importance because it also increases capillary permeability in the uterus feeding the fetus. By contrast, more dramatic increases of permeability increase the risk that the fetus will suffer a miscarriage in the first trimester. This process is reversed in the second and third trimester of pregnancy: androstenediol and 2-methoxyestradiol are increased, antiangiogenesis, antiproliferation as well as Th1 cytokines in the mother are decreased (Figure 8) (30).

Thirty years ago, Schindler and colleagues detected high amounts of androstenediol in the umbilical cord of pregnant women (73, 74), supporting evidence that androstenediol stabilizes the membranes (30). Interestingly, umbilical cells are presently used for screening of plant-derived compounds exhibiting antiangiogenesis (1). It is also of interest that at that same time Thijssen detected the antiangiogenic effects of androstenediol working against breast cancer, revealing that antiangiogenesis is mostly related to antiproliferation (75).

Inflammatory Th1 cytokines have profound effects on endothelial function not only in embryology (Figure 11) but also in regulation of vascular tone, permeability, and leukocyte diapedesis in many diseases (76–78): e.g., during times of overwhelming sepsis, these inflammatory mediators trigger septic shock, a syndrome associated with endothelial cell failure and death (76). Likewise, “successful” tumors also appear to have the capacity to manipulate endothelial function, as they promote their own spread and become vascularized (79, 80).

The endothelium regulates these dynamic interactions with the environment through intracellular signaling cascades. As is the case in many other tissues, JNK- and NF-κB-dependent pathways play an important role during the endothelial response to inflammatory stress (81–84). In resting cells, NF-κB (i.e., RelA/p65 and NF-κB1/p50) associates with IκBα, a negative regulator, forming an inactive complex. Upon stimulation with an appropriate ligand (e.g., TNF-α, IL-1, IL-6), IκBα serines 32 and 36 become phosphorylated, and the protein is targeted for degradation. This releases the p65:p50. Moreover, these studies have revealed the crucial role this pathway plays in antagonizing apoptosis (85). Gene-targeting studies, which have validated some observations about NF-κB function, have been hampered by redundancy.
Figure 15  A conceptual model of cytokines in cancer. Tumor and immune cells are sources of cytokines, which support the growth of cancer and lead to psychobehavioral symptoms (fatigue, depression, and cognitive impairment), drug toxicity, drug resistance, anorexia and cachexia, pain, and cancer recurrence and progression. Genetic background, cancer treatment, and psychological distress can corroborate the production of cytokines. In cancer survivors, hyperactive immune cells might be the major source of cytokines in psychobehavioral symptoms (40).

Antiangiogenic effects are of vital importance to stabilize trophoblasts, umbilical cords, and chimeric cells around existing epithelial cancers. For this reason, Th1 cytokines play an important role during birth or miscarriage, rupture of the uterus membrane, and even as regards heart infarction and stroke (Figures 11 and 12) (36).

Figure 16 Schematic description of crucial steps of cell cycle. Plants inhibit cell cycle in the G2-phase and in the M-phase (mitosis) like the natural hormone 2-methoxyestradiol, which is increased, whereas tamoxifen acts in the G1-phase (47).

Figure 17 Schematic how antiangiogenic compounds can stabilize membranes around a tumor and contains its ingredients. If it is mediated via NF-κB, then Th1 cytokine release and proliferation can also be decreased.

within the family of NF-κB transcription factors and embryonic lethal phenotypes (60, 85–87).

During pregnancy, cells from the embryo can permeate the trophoblast layer and combine with preexisting epithelial cancer cells of melanoma, breast, or colon cancer cells (35). In total, 8% of all cancers are melanoma and receive fetal cells during pregnancy, which can be identified in 68% of all melanoma after pregnancies (35). Lost fetal cells in the epithelium of the mother have high potential to mediate angiogenesis so that preexisting epithelial cancers can grow. They can stay in epithelial layers up to 40 years after birth and are capable of feeding preexisting tumors at any time (35). This was demonstrated by chimeric cells in the epithelial cells of the mothers which expressed the male phenotype of their sons (35).

Antiangiogenesis of soy

Regarding cases involving in vitro, it has been reported that soy isoflavones reduce angiogenesis by reduction of cytokines such as TNF-α and IL-6, via the silencing of the MAP-kinase/NF-κB cascade of immune cells (Figure 2) (88). Tumor angiogenesis plays a pivotal role in the complex, multistep nature of cancer growth and spread. Angiogenesis is intimately involved in metastasis at the site of entry of tumor cells into the vasculature, as well as at the site of eventual metastasis (89). In this regard, a relationship between tumor cell invasion and tumor-induced angiogenesis is described,
with cooperative functions of both processes during tissue breakdown and cell migration (89). Adlercreutz discovered 25 years ago that soy has tremendous antiangiogenic and membrane-stabilizing affects (88). Microarray analysis of gene expression of prostate cancer cells confirmed that soy isoflavones affect more than 1000 genes, and many affect capillary membrane stabilization and angiogenesis (89). Many genes governing angiogenesis resemble gene expression necessary in uterus rupture of pregnant women, such as downregulation of MMP-9 (89). Surprisingly, we found in our own in vitro gene expression studies with fermented soy that although docetaxel is an anticancer drug, it caused upregulation of MMP-9 and downregulation of osteoprotegerin, which could promote cancer cell growth in the bone environment (89).

The antiangiogenesis marker Ki67 was increased in biopsies from tumors in breast cancer patients consuming fermented soy (Figure 18).

Currently, the local tumor compartment is seen as a functional unit composed of two distinctly different cells: cancer cells (Figure 17) and immune cells (Figure 5) (40–46). Immune cells and a network of proinflammatory and anti-inflammatory cytokines collaborate in the development and progression of cancer (46, 90, 91). Cytokines from immune cells as well as cancer cells can leave the local tumor compartment into the peripheral blood stream and spread the disease as well as increase cancer symptoms (Figure 5 and 6). Cytokine profiles (particularly IL-6 or TNF-α) of various cancers in the blood might even prove to be prognostic of cancer outcome (92–99).

There are two different ways to detect efflux of cytokines from tumor sites: first, one can detect cytokine levels in the systemic compartment, and second, one can detect typical symptoms of cancer patients particularly at a later stage of cancer development (Figure 15). Efflux of cytokines from the local tumor compartment is associated with fatigue, depression, and cognitive impairment, and can affect quality of life before, during, and after treatment (Figure 15) (40). Tumor cytokines, despite their large molecular weight, can pass the blood-brain barrier (Figure 15).

Chemotherapy frequently increases side effects in cancer patients and increases cachexia (Figure 19). In a clinical study, during the course of chemotherapy, fermented soy suppressed cachexia (Figure 19) (38). Cachexia in cancer patients is directly related to cytokines in the blood (Figure 15) and increased NF-κB activity of immune cells (Figure 1) (91, 100–107). Cachexia accompanies numerous diseases (AIDS, cancer, rheumatic arthritis) and is a major cause of weight loss, increased mortality, and affects more than 5 million people in the United States alone (106). In total, 30%–60% of people with cancer suffer from fatigue, cachexia, and a subset of patients, especially women with breast cancer, also suffer from cognitive impairment during and after treatment (108–110). Cachexia, fatigue, and cognitive decline have a negative impact on the quality of life and such symptoms can persist for at least 10 years in some breast cancer survivors (110). Soy isoflavones reduced TNF-α in the systemic blood compartment (Figure 14) (38). Our results are corroborated by in vitro studies showing that isoflavones reduce TNF-α release in immune cells (60).

Figure 18  Histology of a breast cancer sample of a 38-year-old woman. Upper row was taken before consumption and lower row was taken 14 days later. ‘‘H&E’’ denotes hematoxylin and eosin. Hematoxylin and eosin stain is frequently used for routine tissue preparation. Eosin is an acid aniline dye. It binds to and stains basic structures (or negatively charged structures), such as cationic amino groups on proteins. It stains them pink. It can be interpreted as DNA activity and the loss of color as a reduced DNA activity. F8 as a vascular marker is discussed by Folkman (79). Ki67 is a common marker of antiangiogenesis. Courtesy of Drs. Karen McCarron and Regina Chorsky.

Figure 19  Influence of daily consumption of a fermented soy formulation on appetite loss (cachexia) in cancer patients under chemotherapy compared to a group receiving placebo solution containing casein. This was a double-blind study. Reproduced from Ref. (39).
Some immune stimulating Th2 cytokines, including interferon-α and IL-2, have been used in cancer treatment; however, they can cause fatigue, depression, and cachexia and other symptoms (111–113). Adjuvant and neoadjuvant treatment of women with breast cancer with paclitaxel also increased serum levels of IL-6, IL-8, and IL-10, and these changes correlated with joint pain and flu-like symptoms (114). Other anticancer drugs known to increase inflammation-causing cytokines include etoposide (115), cisplatin (116), and bleomycin (117).

**Antiproliferative effects of soy isoflavones via NF-κB on circulating tumor cells**

Clearly, the boundaries between regular proliferation, hyperplasia, and cancer, in endometrium, are difficult to define, because they share many similar mechanisms on the genetic level (Figures 10 and 11) (118). These mechanisms are controlled by natural hormones via hormone receptors and hormone receptor independent mechanisms (118). It has been reported that soy isoflavones reduce not only cytokines, such as TNF-α and IL-6, via silencing of the MAP-kinase/NF-κB cascade of immune cells (60, 119), but NF-κB is also silenced in cancer cells (60). Our own in vitro experiments showed strong antiproliferative effects by fermented soy, kinetically, as well as on the molecular oncology level, in breast-, colon-, lung-, prostate- and liver cancer cell lines (Table 1) (120).

For the first time ever, results of in vitro gene expression studies in cancer cell lines of a plant-based formulation (fermented soy) are corroborated by in vivo investigations with CTCs after extraction from blood (Tables 1 and 2). Highly sensitive and specific immunocytochemical and molecular assays now enable the detection and characterization of CTCs at the single cell level in bone marrow or peripheral blood, providing insights into the first crucial steps of the metastatic cascade (6, 121). CTCs, tumor cells leaving the local tumor compartment into bone marrow or peripheral blood, are of utmost clinical importance for the establishment of distant metastasis during the metastatic cascade (121–131). The advantage of CTC diagnosis over cancer cells from a local tumor compartment is first, an easy detection, second, reduces the risk of local efflux of cytokines into the periphery by the needle, and third, they consist of cell forms from which metastasis can develop. Prospects and limitations of this method are discussed in the literature (50, 121).

2-Methoxyestradiol expressed similar anticancer properties like soy isoflavones on the genetic level, such as reducing NF-κB (Figures 20 and 21) (132–134).

In total, 50% of all cancer patients show no sign of tumor suppressor gene p53 expression. Therefore, the increase of tumor suppressor factor p53 by soy (Figure 22) could help to explain prevention of breast cancer by soy, which was viewed as a first- and second-line defense (17, 47). Soy isoflavones ability to reduce NF-κB is not limited to cancer symptoms. Patients suffering from pollen allergy or frequent flu infections reported complete remission of these symptoms (38). It was reported in the literature that intracellular release of specific cytokines in asthma or other allergic reactions is linked to increased c-Jun kinase, p38 MAP-kinase, and NF-κB in local immune cells (135–137). First-generation antihistamines such as diphenhydramine and chlorpheniramine reverse cytokine afforded eosinophil survival by enhancing apoptosis (137). It can therefore be concluded that first-generation antihistamines and soy isoflavones share similar immune modulating responses.

It is particularly important that activated NF-κB mediates the chemoresistance of anticancer drugs, as this is a major problem in cancer therapy (70, 138–145). There is a clinical report, where a prolonged stabilization of platinum resistant ovarian cancer was obtained when consuming fermented soy (146). In the future, plant extracts, such as fermented soy, can help to avoid chemosensitization. Curcumin (140), silybinin (144), and Hibiscus (147) can also improve cancer therapy if used in combination with anticancer drugs by silencing NF-κB.

Our clinical results could also explain why tamoxifen and isoflavones do not interfere with breast cancer protection, as was seen in a large epidemiologic trial, where the combination of tamoxifen with consumption of soy isoflavones reduced the risk of recurrence risk of breast cancer by more than 60% when compared to tamoxifen alone (47). Tamoxifen acts via blocking ER-α. Also, tamoxifen acts on the G1-phase of the cell cycle, whereas isoflavones act on the G2-phase (Figure 16) (148). Therefore, our investigation supports the observation that a combination adjuvant therapy in breast cancer patients can reduce a relapse of breast cancer.

**The immune system of babies does not respond to soy**

Soy-based nutrition formulas for babies have been in use for more than 100 years (149). A committee of pediatricians judged their use as safe (149) and a recent literature review of the US National Institute of Environmental Health Sciences did not reveal anything other than a nutritional effect, without any effect on the immunity or any other physiological effect in babies (150). There is a theoretical concern that ingredients such as soy isoflavones could have negative effects in babies, particularly causing cancer in female babies or feminizing in boy babies (150).

The authors of this review have learned that baby soy formulas have a high soy isoflavone content, exceeding that of natural dietary consumption in Asian food up to 20 times (calculated at 160–960 mg/day for an 80 kg adult male) (150). The question is: why does soy express measurable effects in adults but no effects in babies. The trophoblast theory could help to answer this important question.

As can be seen in Figure 23, androstenediol, which is correlated to immunity (Figure 13), is increased only during childhood and adrenarche and is negligible in babies (151, 152). The reader of this review is encouraged to read the
Table 1 Gene expression changes by fermented soy.

| Gene       | Function                     | Breast cancer | | Prostate cancer | | Ovarian cancer | | Liver cancer | | Lung cancer |
|------------|------------------------------|---------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|            |                              | **In vivo**    | **In vitro**      | **In vivo**      | **In vitro**      | **In vivo**      | **In vitro**      | **In vivo**      | **In vitro**      |
|            |                              | circulating human | cell culture BT474 | circulating human | cell culture (LNCaP) | circulating human | cell culture (SW480) | circulating human | cell culture (HepG2) |
| BAX        | Apoptosis                    | –             | Up                | Up                | Up                | Up                | Up                | Up                | Up                |
| Bel2       | Apoptosis                    | –             | Down              | Down              | Down              | Down              | Down              | Down              | Down              |
| C-myc      | –                            | Down          | Down              | Down              | Down              | Down              | Down              | Down              | Down              |
| ER-α       | Proliferative estrogen receptor | –             | –                 | –                 | –                 | –                 | –                 | –                 | –                 |
| ER-β       | Antiproliferative estrogen receptor | –             | –                 | Up                | Up                | Up                | Up                | Up                | Up                |
| Telomerase | Anticancer marker           | Up            | Up                | Up                | Up                | Up                | Up                | Up                | Up                |
| Cell cycle inhibitor p21 | Inhibits cell proliferation      | Up            | Up                | Up                | Up                | Up                | Up                | Up                | Up                |
| VEGF       | Angiogenesis marker         | –             | –                 | Up                | Up                | Up                | Up                | Up                | Up                |
| MMP-9      | Marker indicating membrane stability | Down         | Down              | Down              | Down              | Down              | Down              | Down              | Down              |

In vitro cell culture experiments compared to in vivo human cancer patients circulating tumor cells after soy consumption (by blood extraction). This Table summarizes in vitro and in vivo effects of fermented soy of gene expression changes. In vivo results were reproduced from Ref. (38). In vitro results were reproduced from Ref. (120). Results from fermented soy experiments are compared to gene expression changes by 2-methoxyestradiol (results were taken from the literature). BT474, human breast cancer cell line; HepG2, human liver cancer cell line; LNCaP, human prostate carcinoma cell line; SW480, human colorectal adenocarcinoma cell line; MMP-9, matrix metalloproteinase; VEGF, vascular endothelial factor.
Table 2  Comparison of Bcl2/BAX ratio.

<table>
<thead>
<tr>
<th>In vitro cell culture cancer cell line</th>
<th>Breast cancer BT474</th>
<th>Prostate cancer LNCaP</th>
<th>Liver cancer HepG2</th>
<th>Lung cancer SW480</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease of Bcl2/Bax ratio</td>
<td>–94%</td>
<td>–52%</td>
<td>–64%</td>
<td>–52%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In vivo, determined in extracted human cancer patient after 3 months of soy consumption</th>
<th>Breast cancer</th>
<th>Prostate cancer</th>
<th>Ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease of Bcl2/Bax ratio</td>
<td>–20%</td>
<td>–94%</td>
<td>–80%</td>
</tr>
</tbody>
</table>

In vivo results were reproduced from Ref. (38). In vitro results were reproduced from Ref. (120). Bcl2/BAX ratio is a surrogate marker for NF-κB. See legend to Table 1 for abbreviations.

review by Loria, describing the relationship between androstenediol, cytokines, and immunity (37), because it becomes apparent that the immunity of babies is simply too low to respond to soy isoflavones via this pathway. The neonate is born with a distinct immune system that is biased against the production of T-helper cell 1 (Th1) cytokines (153). Birth imposes a great challenge on the neonatal immune system, which is confronted with an outside world rich in foreign antigens. Exposure to these antigens shapes the developing neonatal immune system and it takes several years until immunity is fully developed (153). It takes several years inducing Th1 or Th2 polarized responses that could extend beyond the neonatal age and counteract or promote allergic sensitization (153). The immune systems of a mother and the fetus have to be separated and could be a necessity to survive the first three months within the mother. Therefore, soy does not provide any protective effect against allergies or neurodermatitis in babies, owing to diminished Th1 response. However, we observed a rather impressive reduction of allergies in cancer patients after consumption of a fermented soy formulation (38).

Soy isoflavones have no breast cancer protective effects later in life when consumed by babies. The cancer protective effect begins in adrenarche (12). Timing of soy consumption is important (12). As can be seen in Figure 23, androstenediol significantly increases only in childhood. Many hormones are necessary to elicit a complete immune protection in children, but androstenediol blood concentration seems to be a specifically important hormone for vaccination success (37).

One could argue that the androstenediol concentration of the mother will diffuse into the amniotic fluid and can elicit effects on the fetus. However, any effect of androstenediol on the fetus can be excluded, because the fetus HPA axis (androstenediol is a hormone metabolized by the adrenals) and the HPA axis of the mother are completely separated and cannot interfere with each other, as was shown in animal experiments (154).

Therefore, it can clearly be excluded that fluctuations of hormones, which are affecting the immunity of the mother during pregnancy, such as androstenediol (154), 2-methoxyestradiol (31), and Th1/Th2 cytokines (153), will not affect the fetus. By contrast, pediatricians wish that babies would exhibit sensitivity to Th1/Th2 cytokines, which would reduce their risk of suffering from allergies and neurodermatitis (153). However, there seems to be a price to pay for surviving in the womb as a fetus. This corroborates that soy might need a developed immune system, which babies have not yet developed.

Also, soy isoflavones are coined as “phytoestrogens”, leaving a false impression on their pharmacologically efficacy. As was explained earlier, soy might not even need hormone receptors to elicit their pharmacological effects. Also, it is reported that the affinity of soy isoflavones for ER-β is as least 20–30 times greater than their affinity to ER-α, the classical estrogen receptor. If they exhibit significant ER-α activity (155), then perhaps growth in babies could be held back, due to close of the growth plates, a condition that has never been observed. There are other important clinical observations which support that soy has no classical estrogenic effects. As explained above, soy isoflavones are not recognized by the liver during the first-pass metabolism as an estrogen, like estradiol in contraception or in hormone replacement therapy. The difference between ER-β and ER-α is evident when investigating androstenediol, which is structurally similar to soy isoflavones. Both share that they are ER-β-agonistic and not ER-α-agonistic (148). ER-β can be an antiproliferative hormone receptor with strong cancer protective effect (148). Androstenediol, which increases sig-
significantly during adrenarche in both sexes, elicits no estrogenic effects on boys or girls, but is, however, responsible for the strong bone growth during puberty without producing any feminizing effect (151, 152). The later increase in bone mass during puberty by estradiol and testosterone increases bone stability, whereas individuals experiencing adrenarche have the highest risk of suffering from broken bones (151, 152). It is interesting to note that soy isoflavones are assumed to be bone-protective. The effect of androstenediol on bone growth proves that estrogen or testosterone are not needed for bone growth. There could be a general misconception about the physiologic effect of soy isoflavones, which might not be mediated via the cancerous, highly proliferative ER-α.

Discussion

It has been shown that soy substantially affects and alters gene expression with regard to cancer in circulating tumor cells in cancer patients. There are too few patients to make general conclusions. Our results, however, are corroborated by in vitro experiments in cancer cell lines and the literature. Although epidemiological evidence is mounting that soy could be beneficial in cancer protection, the mechanism of action is still not fully understood. For example, it was found that soy isoflavones protect women with ER− tumors in Oriental (14–16) and Caucasian women (17) for which no prevention has yet been established. ER− cells are less differentiated cancer cells, have virtually no hormone receptors...
Therefore, this paper suggests a new mechanism of action, which is based on the “trophoblast theory” as well as the evolving field of cancer stem cell theory.

Soy contains many anticancer ingredients in addition to soy isoflavones (156). Soy isoflavones have strong anticancer activities: they interfere with steroids which are effective in pregnancy to contain highly proliferative cells, because they are structurally similar to androstenediol and 2-methoxyestradiol (Figure 1). These hormones increase apoptosis by increasing NF-κB. The barrier function of the trophoblast has to be decreased to allow nutrients and oxygen to penetrate freely to feed the embryo (Figure 8). This increases the risk that cells from the trophoblast overcome the barrier and can seed in the mother and can be found up to 40 years after pregnancy (35). These cells are highly proliferative and can help preexisting epithelial cancer cells, e.g., in skin and grow to become melanoma, one of the few cancers, which is increased by pregnancy (35) in addition to fibroma (19). During pregnancy, a small number of fetal cells enter the maternal circulation (Figure 7) (35). These cells persist and then migrate to various maternal tissues where they can engraft and differentiate, particularly if there is organ damage, adopting the phenotype of the host organ. Although pregnancy decreases breast cancer risk, it is a concern that young pregnant women suffer from increased melanoma risk, although melanoma normally occurs in older individuals (35). Cells from the embryo are capable of inducing angiogenesis around cells they engulf, so that preexisting tumors can obtain blood supply for their growth providing “fuel” for tumor growth. This could explain why melanoma can be detected in 18 year olds after pregnancy (157).

Therefore, hormones are needed, slowing down cell activity entering the blood circulation of the mother. Hormones slow down cells from the embryo and must have two capabilities: antiangiogenesis as well as an increase of apoptosis to reduce their activity. However, these highly proliferative cells, although they adopt the phenotype of their host environment, are not differentiated and could be considered stem cell-like and have no or insignificant amounts of hormone receptors. Therefore, a “hormone receptor independent mechanism” is necessary to reduce their activity, where pre-existing tumor cells with the highest malignancy can be downregulated via “non-hormone receptor mechanisms” (19). There is developing evidence that hormones, such as 2-methoxyestradiol, are not only effective in pregnancy but are also effective in reducing rheumatoid inflammation (158, 159) as well as cancer in humans (160). 2-Methoxyestradiol has no affinity to hormone receptors, but is, however, highly effective in slowing stem cell-like tumors (Figure 9) (19).

More than 30 years ago, Judah Folkman developed the concept that inhibition of VEGF by targeting therapy could lead to tumor containment by antiangiogenesis of blood capillaries (79). Clinical efficiency of VEGF inhibition was modest in monotherapy (7, 8) and reduction of capillaries in tumors can cause failures of chemotherapy to work (7, 8). Jain reinvestigated this concept and came to a different conclusion (7, 8). Blood flow reduction in tumors could be an important mechanism for cancer control; however, an important shift in antiangiogenesis research is necessary. The old concept “reduction” of capillary formation (destroy and kill) has to be replaced with stabilizing abnormal, leaky existing vessels in cancerous tissues which makes them less leaky (7, 8).

Stabilizing and destabilizing membranes in cancer and pregnancy is very similar (Figure 7). As outlined, cytokines created by tumor cells make tumor vessels leaky to oxygen, nutrients, and most of all cancer cells and cytokines that cause cachexia, depression, and vomiting are similar to cytokines, which are increased in the first trimester of pregnancy (36). Th1 cytokines are increased in the first trimester of pregnancy, and many women feel a reduction in their well-being (Figure 8) (36). Capillary stability and a decreased permeability are of vital importance for a successful pregnancy (Figure 11) and can also serve as a model for cancer “containment”. Hormones modifying capillary permeability show not only antiangiogenic but also antiproliferative properties. Antiangiogenesis is combined with antiproliferative activity and is mediated by NF-κB, like two sides of a coin. Therefore, antiangiogenesis in vivo is always combined with apoptosis via NF-κB.

A circadian rhythm of estradiol uptake was detected in transdermal delivery in postmenopausal women, although estradiol was delivered constantly by zero-order release kinetics (161, 162). We assumed at that time that estradiol uptake, naturally through capillaries in the ovary and transdermally by skin capillaries, is influenced by the circadian rhythms of Th1 cytokines (162). Clinicians can observe circadian rhythms of Th1 cytokines in their patients suffering from fever, fatigue is increased in the afternoon, a common side effect of Th1 cytokines.

Almost 30 years ago, Schindler and colleagues discovered high levels of androstenediol in capillaries of umbilical veins of pregnant women (73, 74). Androstenediol is structurally and pharmacologically similar to soy isoflavones (Figure 1). Interestingly, the umbilical vein cell model is presently used to screen for antiangiogenic drugs in cancer treatment (35).
The textbook knowledge of antiangiogenic effects of soy (89) is correlated to a decrease of Th1 cytokines (Figure 14). Increased Th1 cytokines increases the risk of miscarriage (rupture of the uterus membrane). There are now reports that miscarriage risk can be lowered by plant formulations, reversing Th1/Th2 disbalance (163). Increased Th1/Th2 cytokine balance alters barrier functions in many diseases, not only in miscarriage but also for heart infarction, stroke, and cancer (Figure 11) (36).

Soy isoflavones are structurally similar to androstenediol and 2-methoxyestradiol (Figure 1). Soy increases 2-methoxyestradiol by approximately 40% in humans, owing to the fact that they compete for the same enzyme (164). Therefore, in the in vivo situation it could be difficult to distinguish between the effect of soy isoflavones and 2-methoxyestradiol. 2-Methoxyestradiol is currently in clinical trials to treat breast cancer (160). Colchicine and 2-methoxyestradiol exhibit antimitosis activity (Figure 1). In addition to apoptosis, antiangiogenesis, a chemotherapy-like mechanism is also used to protect the mother. Therefore, a combination of chemotherapy with soy formulation should be tested in the future, because mechanistically, antimitosis, apoptosis, and angiogenesis do not interfere with each other. This should be corroborated in larger trials.

**Outlook**

In addition to cancer protection of soy products, hundreds of millions of people suffer from non-cancerous conditions of abnormal vasculature (7, 8). Blood vessel repair beyond cancer could be a key component of the therapeutic arsenal for those diseases (7, 8). Our studies can lead to other applications in addition to cancer, such as stroke, heart infarction, and mental diseases. Our findings need to be corroborated by larger studies in cancer therapy. The trophoblast theory of cancer might not only explain some of the cancer protecting effects of soy but it could also explain why soy has no hormonal effects on babies consuming soy nutritional formula, and also explain some pharmacological effects such as bone protection.

**References**


associated antigens is related to estrogen receptor status. Cyto-
98. Benoy IH, Salgado R, Van Dam P, Geboers K, Van Marck E, Scharpè S, Vermeulen PB, Dirix LY. Increased serum interleu-
kin-8 in patients with early and metastatic breast cancer cor-
rrelates with early dissemination and survival. Clin Cancer Res
100. Ramesh G, Reeves WB. TNF-α mediates chemokine and cyto-
proinflammatory cytokine production and sickness behavior-
like symptoms in a mouse model of cancer chemotherapy-relat-
proinflammatory cytokine production and sickness behavior-
like symptoms in a mouse model of cancer chemotherapy-relat-
105. Ferrucci L, Penninx BW, Bandeen-Roche K, Balfour J, Leveille SG, Fried LP. Change in muscle
108. Vardy J, Tannock I. Cognitive function after chemotherapy in long-term survivors of breast cancer and lym-
farb PM. Neuropsychologic impact of standard-dose systemic
chemotherapy in long-term survivors of breast cancer and lym-
111. Bower JE, Ganz PA, Bernards C, Rowland JH, Meyerowitz BE, Belin TR. Fatigue in long-term breast carci-
113. Capuron L, Ravaud A, Dantzer R. Timing and specificity of the cognitive changes induced by interleukin-2 and interferon-
114. Tsavaris N, Kosmas C, Vadiaka M, Kanelopoulos P, Boula-
matsis D. Immune changes in patients with advanced breast
proinflammatory cytokine production and sickness behavior-
like symptoms in a mouse model of cancer chemotherapy-relat-
116. Ramesh G, Reeves WB. TNF-α mediates chemokine and cyto-
119. Ramesh G, Reeves WB. TNF-α mediates chemokine and cyto-


161. Rohr UD, Saeger-Lorenz K. 17β-Estradiol matrix patch remov-