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Phytoestrogens and Breast Cancer Risk

Phytoöstrogene und Brustkrebsrisiko

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Abstract

Breast cancer is the most frequently diagnosed form of cancer among women worldwide with incidence and prevalence rates still rising. The most common subtype of breast cancer is related to estrogen receptor stimulation and standard therapeutic interventions thus act through blocking this pathway. At the same time, estrogenic diets are supposed to act protective against breast cancer development, a conclusion derived from the lower incidence of breast cancer in Asian countries where the consumption of estrogenic substances far exceeds that of Western countries. A huge number of herbal mixtures applied in TCM also contain estrogenic substances, and some of these have even been applied to treat breast cancer patients. Importantly, recent studies reported that some phytoestrogens contained in TCM formulations may seriously interfere with established cancer drugs, indicating potential detrimental effects when applied in the wrong context. In the present study I thus summarize our current knowledge on the role of estrogens in breast cancer development, compare epidemiological studies evaluating pros and cons of estrogenic diets, and highlight some important mechanistic studies on the interaction between estrogenic substances and breast cancer drugs. This overview shows that epidemiological investigations produced often contradictive results, whereas mechanistic studies raise considerable concern regarding interfering estrogenic drugs, leading to the recommendation to refrain from the use of TCM mixtures with estrogenic substances for breast cancer patients until less equivocal insight is presented by future studies.

Zusammenfassung

Brustkrebs ist die weltweit meist-diagnostizierte Krebsform bei Frauen und weist noch immer zunehmende Inzidenz- und Prävalenzraten auf. Der am häufigsten auftretende Subtyp von Brustkrebs ist ursächlich mit einer Aktivierung des Östrogenrezeptors verbunden, weshalb gegenwärtige Standardtherapien vor allem über eine Blockade dieses Signalweges wirken. Andererseits wird der Aufnahme östrogenhaltiger Nahrung eine protektive Wirkung gegen die Entstehung von Brustkrebs zugeschrieben, da dieser in asiatischen Ländern mit wesentlichen höherer Zufuhr von östrogenwirksamen Substanzen durch die Nahrung eine bedeutend niedrigere Inzidenzrate aufweist. Eine große Anzahl von Kräutermischungen, die in der TCM angewandt werden, enthält

östrogenwirksamen Substanzen und einige davon werden sogar spezifisch zur Behandlung von Brustkrebspatientinnen eingesetzt. Allerdings zeigen einige rezente Studien, dass bestimmte östrogenwirksame Substanzen aus TCM-Rezepturen mit etablierten Krebsmedikamenten interferieren und deren Wirkung signifikant einschränken können. Dies deutet darauf hin, dass solche Substanzen potentiell schädliche Auswirkungen haben können, wenn sie inadäquat und ohne ausreichendes Wissen angewandt werden. In der vorliegenden Arbeit habe ich daher unseren gegenwärtigen Wissensstand über die Bedeutung von Östrogenen bei der Entstehung von Brustkrebs zusammengefasst, epidemiologische Studien hinsichtlich Pro und Kontra östrogenhaltiger Ernährung in Bezug auf Brustkrebs verglichen und wesentliche mechanistische Untersuchungen zur Interaktion zwischen östrogenwirksamen Substanzen und Brustkrebsmedikamenten besprochen. Dieser Überblick zeigt, dass die epidemiologischen Studien häufig widersprechende Befunde ergaben, während die mechanistischen Studien ernsthafte Bedenken gegen den Einsatz östrogenwirksamer Substanzen erheben. Daraus wird die allgemeine Empfehlung abgeleitet, beim derzeitigen Wissensstand von der Anwendung solcher Substanzen, wie sie in TCM-Rezepturen enthalten sind, Abstand zu nehmen, bis eindeutiger Erkenntnisse vorliegen.

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Abbreviations:

ER	estrogen receptor
HRT	hormone replacement therapy
PR	progesterone receptor
HER2	Human epidermal growth factor receptor 2
CDK	cyclin-dependent kinase
TCM	Traditional Chinese Medicine
BRCA1/2	Breast Cancer gene 1/2

I. Introduction

Traditional Chinese Medicine (TCM) has become increasingly popular in the Western world over the past decade and the demand for a more holistic approach putting the individual patient rather than the disease into the focus of attention has led to a broader offer of TCM-based treatments covering a wide range of application, including cancer therapy (Efferth et al., 2007; Hsiao and Liu, 2010). Patients are offered TCM-derived medicine for disease prevention and for curative purposes, either alone or as complementing standard Western medical measures primarily based on treatment with drugs. In many instances TCM-based measures involve the provision of traditional herbal mixtures or plant ingredients often containing, among others, natural anti-cancer compounds such as camptothecin derivatives or vinca-alkaloids (Wang et al., 2012). In addition, since the much lower incidence of certain diseases and cancer types in Asian countries has been linked with their quite different dietary habits (Adlercreutz, 2002; Adlercreutz and Mazur, 1997), in particular with the much higher intake of soy bean-derived food products (Messina et al., 2006), soy products and their active ingredients have received a lot of attention, the latter including phytoestrogens. These substances are also main constituents of medicine provided for the treatment of menopausal problems and for various other conditions, as can be seen from overviews published in Chinese journals (Huang and Yu, 2016; Yuan et al., 2012). However, endogenous estrogens and phytoestrogens have been linked with breast cancer risk (Samavat and Kurzer, 2015; Wen et al., 2017; Yager and Davidson, 2006). Many studies have suggested that a high intake of phytoestrogens is beneficial, suggesting that the administration of Chinese herbal mixtures could exert protection against breast cancer development or support breast cancer therapy. On the other hand, there is some evidence that phytoestrogens may even enhance, rather than reduce, breast cancer risk and that compounds derived from phytoestrogens may counteract Western drug-based therapy of breast cancer (Patisaul and Jefferson, 2010). This leaves the Western practising physician who is open to implement the benefits of the TCM approach into treating cancer patients, and specifically women with breast cancer, at loss, as it remains unclear if this is beneficial or detrimental for the patient.

The aim of the present work is therefore to provide a comprehensive overview on this issue, discussing breast cancer and its treatment, the role of estrogens in the development of breast cancer and the potential role of phytoestrogens in this scenario.

For this purpose, the scientific literature on either beneficial or adverse effects of the intake of high levels of phytoestrogen-containing food will be reviewed, discussing some exemplary epidemiological and various mechanistic studies.

II. Breast cancer: incidence and epidemiology

Breast cancer is the 2nd most common cancer type worldwide after lung cancer (excluding non-melanoma skin cancer) and the most frequently diagnosed form of cancer among women in 140 of 184 countries worldwide (Stewart and Wild, 2014; World Cancer Research Fund, 2018). Its incidence rate has dramatically increased over the past decades such that breast cancer now accounts for 25% of all cancers in women. In 2012 this amounted to 1.7 million new cases diagnosed (Breast Cancer Research Foundation, 2018) and in 2018 this figure has risen to 2 million new breast cancer diagnoses (World Cancer Research Fund, 2018). Both incidence rates and survival rates show large variability worldwide. Thus, incidence in 2012 amounted to 27 in 100.000 in Middle Africa and Asia, while it was 92 in 100.000 in Northern America (World Cancer Research Fund, 2018) and 109 in 100.000 in the EU (Ferlay et al., 2013). Since breast cancer risk increases with age and roughly doubles each decade up to menopause, the increase can at least in part be attributed to increased life expectancy. In addition, aspects relating to Western life style were found to correlate with breast cancer incidence rates. For example, enhanced alcohol consumption was shown to elevate the risk for breast cancer (Frydenberg et al., 2015), as was overweight and obesity and a lack of physical activity (Chlebowski, 2013; McTiernan, 2018). Further, low parity, a higher age at first full-term birth, and reduced periods of breast feeding contribute to higher incidence (Kobayashi et al., 2012). Survival rates have increased over the last years, particularly in Western countries, due to earlier detection because of screening programs, better treatment options at the early, localized stage, and because of generally improved treatments. Five-year survival rates thus amount to 80-90% for women diagnosed with Stage I/II (small tumors or limited local spread to nodes under the arm) in most countries (and up to 99% in the U.S), but amount to only 24% (27% in the U.S) for Stages III/IV (larger tumors or more distant spread beyond the breast or to distant organs) (World Cancer Research Fund, 2018). Overall, as a result of increased incidence rates and better treatment options prevalence of breast cancer is increasing.

Therefore, despite of better survival rates, it is still the main cause of cancer-related death in women worldwide and continues to be a significant society burden, particularly in Western countries.

III. Risk factors

Like many other cancers, breast cancer is a very heterogenous disease and thus there are many causes leading to breast cancer and various forms in which it appears. Risk factors relating to the disease are age, personal and family history of breast disease, genetic predisposition, and several environmental factors (Table 1). Age, as mentioned above, shows a clear-cut relation with a dramatic increase over lifetime up to menopause. This may best be illustrated with statistics indicating that an American woman holds a risk of developing breast cancer of 1 in 202 at the age below 49, and of 1 in 26 between 40 and 59 years of age. Between 60 to 69 years it remains at 1 in 28 (Siegel et al., 2013). Personal and family history involve both other diseases affecting the breast as well as previous experience of breast cancer, i.e. developing a second breast cancer. Genetic predisposition conveys a wide range of risk enhancement, depending on which gene is affected. E.g., a homozygous mutation of DNA-damage repair gene ATM or the tumor suppressor gene CHEK2 confer a 20-40% lifetime risk of breast cancer, while with BRCA1 or BRCA2 mutations lifetime risk is between 40-85% (Shah et al., 2014). Different from these, lifestyle-related risk factors represent a modifiable risk and are estimated to account for 21% of the mortality related to breast cancer (Danaei et al., 2005). These factors include alcohol consumption, which positively correlates with enhanced risk, and physical activity, which exerts a slight and dose-dependent protection amounting to 2-5% risk reduction (McTiernan, 2018; Wu et al., 2013). In line and possibly related with the latter, it was also shown that body mass, and specifically obesity in post-menopausal women is positively related with breast cancer risk (Lahmann et al., 2004; McTiernan, 2018). Radiation exposure is another risk factor, the most probable reason for which is exposure during childhood cancer treatments.

Finally, and in some instances closely intertwined with already mentioned risk factors, hormonal aspects are of pivotal importance, particularly the exposure to estrogen. Accordingly, early menarche reportedly increases the risk to develop an estrogen receptor (ER)-positive tumor almost twofold, whereas a delay in menarche appears to

be somewhat protective (Hsieh et al., 1990; Ritte et al., 2012). Further, women who never give birth to a child show an enhanced risk compared to women experiencing their first birth at 20 or 25, and a slightly reduced risk in comparison to first mothers at 35 (Rosner et al., 1994). Breast feeding, presumably by delaying the return of regular ovulatory cycles and a reduction of endogenous sex hormone levels, confers protection against developing breast cancer and this appears to be correlated with the duration of breast feeding (Rosner et al., 1994). Fittingly, enhanced levels of endogenous sex hormones and, in post-menopausal women high testosterone increase the breast cancer risk (Sieri et al., 2009), and the age of onset of menopause is also positively correlated with enhanced risk (Hsieh et al., 1990; Kelsey et al., 1993).

Table 1: The most important risk factors associated with breast cancer development (modified after Shah et al., 2014).

Type of risk factor		
General	**Hormone-related**	**Life-style related**
Age	Early menarche	Alcohol consumption
Personal history	Parity and age at first full-term pregnancy	Physical activity
Breast pathology	Breast feeding	Obesity
Family history	Testosterone level	Radiation exposure
Genetic predisposition	Age at menopause	

A widely debated and apparently highly complex issue is the impact of hormone replacement therapy (HRT). This treatment which aims at reducing discomfort in post-menopausal women resulting from altered endogenous hormone production makes use of estrogen or combined estrogen and progestogen administration. Over decades of use of this treatment, evidence has accumulated that HRT may increase the risk of breast cancer (Chlebowski and Anderson, 2012). However, a more detailed look into this phenomenon revealed that this may only be true if HRT is used for longer than 10 years (Collaborative Group on Hormonal Factors in Breast Cance, 1997). Further, the risk is enhanced when HRT is started soon after menopause, but less so when initiated at a

later point (Chlebowski et al., 2013). It was also shown that the combined administration of estrogen and progestin increased cancer risk in post-menopausal women with intact uterus and then delayed cancer detection, resulting in enhanced mortality. In contrast, administration of estrogen alone to post-menopausal women with previous hysterectomy reportedly caused a significant reduction of breast cancer risk (Anderson et al., 2003), although a different study suggested unopposed estrogen to increase cancer risk (Colditz and Rosner, 2000). Thus, the importance of hormonal status is beyond doubt, but the details await further clarification.

IV. Breast cancer types and their treatment

Several different subtypes of breast cancer can be discerned. Using different classifications, breast cancers can be grouped according to their invasive characteristics, their site of occurrence, their histological appearance, or the expression of molecular markers. As to the latter, following the most recent guidelines defined at the 2015 St. Gallen Consensus Conference and the recommendations provided by the European Society for Medical Oncology (ESMO) four surrogate intrinsic subtypes are referred to as Luminal A, Luminal B, HER2 (Human epidermal growth factor receptor 2) overexpression, and Basal-like (Senkus et al., 2015) (Table 2). The Luminal A subtype is positive for the expression of ER and progesterone receptor (PR), but negative for the expression of HER2, a receptor involved in the regulation of cell growth, proliferation, differentiation, survival, and angiogenesis. In addition, Luminal A has a low expression level of Ki67, a nuclear protein associated with cell proliferation. Luminal B is characterized as ER- and PR-positive, but can be both HER2-positive or negative, and has a somewhat elevated level of Ki67 expression. In HER2 breast cancer HER2 is enriched, while it is ER- and PR-negative, and in the Basal-like subtype none of these receptors is expressed. Associated with the expression levels of these markers, the different cancer subtypes have different prognostic outlooks ranging from good to poor (Table 2). The subtypes can also differ in the relative expression levels of proteins such as the transcription factor and proto-oncogene MYC, or the expression of Claudins, members of the family of tight junction proteins, and have found to display characteristic metabolic features (Kulkoyluoglu-Cotul et al., 2018). The variation of

Claudin expression levels has led to the discrimination of an own breast cancer subtype (Velloso et al., 2017).

Table 2: Breast cancer subtypes (modified after Velloso et al., 2017 and Kulkoyluoglu-Cotul et al., 2018).

Subtype	Molecular markers	Prevalence	Prognosis
Luminal A	ER^+, PR^+, $HER2^-$, low Ki67	30%	Good
Luminal B	ER^+, PR^+, $HER2^-$ or - $HER2^+$, high Ki67	20% ($HER2^-$) 10% ($HER2^+$)	Poor to intermediate
HER2	ER^-, PR^-, $HER2^+$	15%	Poor
Basal-like	ER^-, PR^-, $HER2^-$ (triple negative)	10%	Poor
Claudin-low	ER-, PR-, HER2-, low Claudin	10%	Poor

ER, estrogen receptor; PR, progesteron receptor, HER2, Human epidermal growth factor receptor 2.

Breast cancer subtypes are also classified according to the site of their occurrence as *in situ* versus invasive breast tumors, accounting for 20% and 80%, respectively. In addition, the former type is grouped into lobular carcinoma *in situ* (20%) and ductal carcinoma *in situ* (80%), up to half of which can progress to become invasive.

Depending on the size and location of the primary tumor and the number of lesions and lymph nodes involved, the expression of the above-named markers is critical for the choice of the treatment strategy applied. Besides preoperative/neoadjuvant chemotherapy, surgical measures of removing tumorous tissue and involved lymph nodes where applicable, and post-operative radiation therapy, adjuvant systemic therapy is a standard component of most breast cancer therapies. For this purpose, three main types of medications are applied, i.e. hormone blocking agents, chemotherapeutics, and monoclonal antibodies.

Following the guidelines defined at the 2015 St. Gallen Consensus Conference, all luminal cancers should be treated with endocrine therapy, i.e. hormone blocking

therapy. At present, endocrine therapy is considered the most efficient first-line therapy for the treatment of ER-positive metastatic breast cancer tumors. Since these tumors require estrogen for their growth, the aim of this therapy is to block ER activation. To this end, the ER can be blocked using the pro-drug Tamoxifen, which in the liver is converted to the metabolites afimoxifene and endoxifen which bind to the ER with high-affinity. This drug may be administered for 5-10 years and is not only widely applied for the therapy of already diagnosed cancers, but has also been approved for the prevention of breast cancer for women belonging to a high-risk group (Powles et al., 2007). An alternative way to prohibit ER stimulation is by preventing estrogen synthesis, using aromatase inhibitors anastrozole or letrozole (Finn et al., 2015; Finn et al., 2016), but these appear to only work for women in post-menopause.

Chemotherapy is primarily applied in combination with endocrine therapy, for the treatment of advanced cancer stages, and for ER-negative breast cancer. Common drugs applied are cyclophosphamide, taxanes and anthracyclines (e.g. doxorubicin), all of which are DNA damaging drugs which thus primarily effect actively proliferating cells. A rather recent addition are drugs affecting cell cycle regulators cyclin-dependent kinases (CDK) 4 and 6 (Dhillon, 2015; Johnson et al., 2016), which together with aromatase inhibitors were found to be extremely effective against specific breast cancer subtypes and which is thus considered a ground-breaking clinical novelty (Wolff, 2016). Due to their mechanism of action these chemicals may exert multiple side effects, including a suppression of immune function and even a damage of heart muscle, and therefore drugs of this types are typically used for only limited periods of 3-6 month.

HER2-positive and hormone receptor-negative cancers are also treated with chemotherapy, but a treatment specifically targeting HER2-positive cancers and usually combined with chemotherapy is application of the HER2-specific monoclonal antibody Trastuzumab. This antibody appears to slow cell growth upon binding to the HER2 receptor and is currently used for approximately one year (Balduzzi et al., 2014).

Depending on multiple additional parameters such as cancer progression and disease burden, the specific expression of other markers, the initial response to any treatment, and the involvement of the individual patient, therapeutic approaches may be combined and specifically adapted.

However, it should be clear from the discussion so far that estrogens play an important role in a large subgroup of breast cancers. Thus, a closer look at estrogen function in general and also in the context of cancer development seems to be justified.

V. The biological functions of estrogens

Estrogens are the main female sex hormones and belong to the class of steroid hormones. Estrogens are primarily produced in the ovaries and in the placenta, and to a small part also in the adrenal glands, in liver, pancreas, bone, breast, brain, skin, adipose tissue, and, in males, in the testicles. According to their main function in females, production of the hormones is linked with the menstrual cycle and is functionally responsible for the development of the female reproductive system and its regulation during pregnancy (Levitz and Young, 1977). Like other steroid hormones, estrogens can easily permeate the cell membrane and then bind to cytosolic and/or nuclear ERs, the main types of which are ERα and ERβ. Both receptor types are encoded by separate genes and can form homo- and heterodimers. Upon binding of estrogen to its receptor, the receptor dimerizes, binds to a hormone-response element of the DNA, recruits some additional proteins to the receptor/DNA complex and ultimately induces the transcription of specific DNA products. The ER controls the expression of a multitude of target genes, the exact nature of which is determined by the tissue expressing the receptor. The complexity of these targets is enhanced by the fact that the ER may not only directly bind to estrogen-response elements on the DNA but can also interact with other transcription factors and their binding to DNA (Enmark and Gustafsson, 1999).

Besides these classical nuclear ER receptors, there are also membrane estrogen receptors. These do not mediate transcriptional responses but modulate intracellular signaling cascades and/or ion channel activities (Soltysik and Czekaj, 2013).

On a macroscopic scale, among the many biological functions of ER signaling are the control of female pubertal development which includes breast development and the maturation and maintenance of the female reproductive system. In addition, estrogens are involved in the control of brain function and behavior (Ogawa et al., 2018), affect the growth of bones and the maintenance of bone mineral density (Khosla et al., 2012), play a role in cardiovascular function (Murphy, 2011), and in the immune system (Yakimchuk et al., 2013) (Table 3).

Within the context of breast cancer development, the role of ER signaling in the control of energy metabolism appears of pivotal importance. This function seems to be an ancient function of the receptor, an assumption not only reflected by its universal expression in all vertebrates, but also in some invertebrates lacking sexual reproduction (Markov et al., 2017). Hence, ER signaling was shown to affect the expression and activity of transporters involved in the metabolism of glucose, amino acids, and lipids in a way that allows cancer cells to rapidly grow and proliferate by both utilizing and shaping their own microenvironment to their advantage (Kulkoyluoglu-Cotul et al., 2018). The exact nature of the many metabolic elements being up- or down-regulated appears to be a breast cancer subtype-specific feature, providing characteristic metabolic signatures to the different subtypes discerned.

Table 3: The most important functions of estrogens

Biological process affected	**Specific effect**
Female pubertal development	Expression of secondary sex characteristics (breast development, hip widening, fat deposits)
Female reproductive system	Maturation and maintenance of the vagina and uterus, maturation of ovarian follicles
Brain function	Control of the libido, cognition, mental health
Bone and skeletal system	Growth at puberty, closure of the epiphyseal plate, bone mineralization, bone density
Immune system	Anti-inflammatory action

VI. The role of estrogens in breast cancer development

Among the different risk factors for breast cancer development, many appear, in one way or another, to be related to estrogen. Thus, the level of endogenous estrogens, the concentration of intermediates of estrogen metabolism, the time of exposure to the hormone, and alterations in ER signaling have been linked with breast cancer risk (Samavat and Kurzer, 2015; Wen et al., 2017; Yager and Davidson, 2006). In line, changes in the activity of enzymes involved in estrogen metabolism, of constituents of

estrogen signaling pathways, and mutations of genes encoding for enzymes and receptors have been examined and appear to correlate with altered risk for breast cancer development.

Mechanistically, the role of estrogen and its metabolites in the initiation of cancer have been relatively well studied. The synthesis of estrogens, like that of all other steroid hormones, originates from cholesterol which is metabolized to the sex hormone through a number of enzymatic steps. Once formed, estrogens may be reversibly interconverted and further metabolized through one of several irreversible hydroxylation pathways involving classical drug metabolization and detoxification enzymes of the Cytochrome P450 family of enzymes. In addition, estrogens and related intermediates may also be conjugated with glucuronic acid and sulfate to make them more water soluble for excretion. Some of the products of these interconversion and detoxification pathways, specifically products of the hydroxylation pathways, have been found to act cancerogenic, although this can clearly not be stated for all of them. Thus, intermediates of the 2-hydroxylation pathway reportedly inhibit cell growth and proliferation in the MCF7 breast cancer cell model and lack tumorigenic activity (Gupta et al., 1998; Schneider et al., 1984). In contrast, products occurring along the 4-hydroxylation pathway were shown to possess carcinogenic potential. For example, the 4-hydroxylated catechol intermediate of estrogen metabolism may form depurinating DNA adducts (i.e. it covalently binds to DNA and removes the purine or pyrimidine base from the DNA double strand) and thereby generate mutations that can initiate breast cancer development (Cavalieri et al., 1997). In line, women bearing a high risk of developing breast cancer had high levels of estrogen-DNA adduct in serum and urine (Gaikwad et al., 2009; Pruthi et al., 2012), making these adducts potential predictive biomarkers. Further, in samples from human mammary fibroadenoma and adenocarcinoma the generation of the 4-hydroxy intermediate was significantly higher than that of the 2-hydroxy product, elevating the ratio of these products compared to samples from healthy tissue (Liehr and Ricci, 1996). Similarly, products of the 16-hydroxylation pathway were observed to induce unscheduled DNA synthesis, reflecting DNA repair in response to DNA damage, and to support anchorage-independent growth in mouse mammary epithelial cells, a hallmark of cancer cells with metastatic potential (Bradlow et al., 1986; Suto et al., 1993; Telang et al., 1992). Further, 16-hydroxyestrone in the urine of experimental animals reportedly reflected enhanced mammary cell

proliferation (Suto et al., 1993; Telang et al., 1992), expression of the Ras oncogene (Suto et al., 1992) and correlated with mammary tumor incidence in mice (Bradlow et al., 1985).

As a corollary, some of these intermediates appear to be involved in enhanced formation of reactive oxygen species (ROS). Particularly catechol-estrogens may be reduced to semiquinones and re-oxidized again, and this redox-cycling may result in the production of super-anion radicals and hydrogen peroxide (Cavalieri et al., 2006). As many other redox-active substances, estrogens can thereby elevate ROS to levels eventually increasing genomic instability (Tian et al., 2014) and contribute to mutations of mitochondrial DNA and in consequence change the expression and function of important constituents of the mitochondrial respiratory chain components as has been observed in breast cancer cells (Tan et al., 2002). An *in vitro* study using the MCF-10A breast cancer cell model provided direct evidence for this described scenario. Thus, it was shown that the exposure of these cells to the redox-active intermediate 4-hydroxyl-estrogen caused their malignant transformation, whereas the concurrent treatment with radical scavenging measures prevented this conversion (Okoh et al., 2013).

Besides their damaging effects on cell proteins and DNA, elevated ROS levels can also act on cell signaling and thereby contribute to carcinogenesis. In particular the excessive activation of PI3-kinase/AKT signaling, an important pathway involved in cell growth, proliferation and migration, was reported to play a decisive role in the induction of malignant transformation of healthy breast cancer cells exposed to the estrogen intermediate 4-hydroxy-estradiol (Okoh et al., 2013). In addition, ROS-induced signaling related to NFkB, a transcription factor controlling cell proliferation and cell death, was linked with anchorage-independent growth of human mammary epithelial cells caused by estrogen intermediates (Park et al., 2009).

Finally, a number of mutations related to estrogen signaling and/or metabolism are considered to be causally related with breast cancer development. Linked with the enhanced production of mutagenic and ROS-enhancing intermediates described above, some polymorphisms of the CYP1B1 encoding gene, a member of the Cytochrome P450 family of enzymes, were reported to produce higher levels of 4-hydroxy intermediates and hence elevate the risk of breast cancer (Hanna et al., 2000). Similarly, a single nucleotide polymorphism in the gene encoding the estrogen-detoxifying

enzyme COMT was linked with a significant reduction in its activity, which in consequence increases the levels of redox-active quinone-intermediates and was thus reported to enhance the breast cancer risk in the Chinese population (Tian et al., 2014; Wan et al., 2014).

VII. Carcinogenesis and development of breast cancer: East versus West

The Eastern view

Traditional Chinese Medicine (TCM) has dealt with cancer since more than 2000 years and has developed concepts for prevention, diagnosis and treatment of the disease (Liu et al., 2015). Besides the description of symptoms, prognosis and differential diagnosis, TCM already provided summaries of supposed cancer pathogenesis and treatment strategies, including suggestions on specific causes such as exo-pathogens, environmental factors, emotional maladjustment and dietary problems. The first direct reference to cancer in a modern sense, using the Chinese word Ai denoting malignant carcinoma, dates back to a book from around 1300 BC, called "*Wei Ji Bao Shu"* (Hsiao and Liu, 2010). Chinese physicians stressed that the main causes of cancers originate from the inside, tumors reflecting the consequences of a systemic disease. Accordingly, prevention of cancer, treatment of the acute disease and chronic treatment combine partial and systemic therapy, the *latter* aiming at strengthening body resistance, eliminating pathogens, and regulating emotion. In other words, rather than trying to eliminate the tumor and/or killing the cancerous cells, the TCM holistic approach tried to modulate the whole body and to increase the quality of life of the patient. Quoting "*Indispensable Medical Reading"* this approach suggests cancer treatment in different stages (Liu et al., 2015): "In the early stage of the disease, the vital qi is strong, the evil qi is light and easily attacked; in the middle stage, the evil qi is deeper, the vital qi becomes weak and should be attacked or benefit; in the end, the evil qi is strong, the vital qi is weaker and should be well benefit." In recent years, the interest in TCM has increased also in the Western world, as numerous drugs long used in TCM were proven to be supportive in the treatment of many diseases (Hempen et al., 2018), including

various forms of cancers. Well known TCM-derived products applied in cancer chemotherapy include camptothecin, vinblastine and vincristine, which are used to treat leukemias, ovarian, lung and colon cancers. These efforts are strongly supported by the Chinese government so as to modernize and economically exploit TCM (Efferth et al., 2007).

As to the pathogenesis of breast cancer, the view of TCM has been described in detail by Schmit (Schmit, 2009), and shall be briefly summarized here. According to this paper, the ancient Chinese name signifying breast cancer is *ru yan*, *ru* denoting the breast and *yan* a rock, which together reflects the occurrence of a solid lump of tissue in the breast. The pathogenic mechanism leading to the development of these lumps involves premenstrual tensions and fibrocystic breast diseases. Emotional imbalance appears to play a particularly important role in this context, with emotional stress, frustration and depression affecting the liver and bringing the flux of qi to a halt. As a consequence of qi stagnation and accumulation heat is produced and a counteracting flow of negative qi is induced. The stagnant qi attacks spleen and stomach, leading to a suggested link between breast cancer and spleen pathology in TCM. As the stagnant flux of liver qi caused by frustration and anger affects the spleen, the latter can no longer fulfil its transporting and transforming function, and once this affects the flow of blood, blood stasis occurs. This again causes the formation of phlegm and rheum, pathological substances which in a negative feedback cycle further aggravate the situation by blocking meridians and vessels. Overall, the impaired flux of qi may eventually affect the specific meridian *Chong Mai*, which runs through the breast and stomach and liver, the latter of which specifically supplies the mamilla. Other parameters such as toxic exposure may add to the situation and combined with the reduced flux of qi this may then cause neoplastic developments. Alternatively, a weakness of the *Zheng Qi* has been suggested to underlie the formation of neoplasia, the *Zheng Qi* in this context reflecting the equivalent of the immune system. This weakness leads to the formation of phlegm, blood stasis and an improper distribution of nutrients.

A summary of TCM approaches towards the treatment of breast cancer has been given by Cohen et al. (Cohen et al., 2002), but a more comprehensive assessment of the role of phytoestrogens playing a particularly important role in TCM in this context will be provided in subsequent chapters.

The Western view

A similar model of different stages but enriched with much more analytical detail is characteristic of the Western view of carcinogenesis and specifically the development of breast cancers. Following the seminal paper by Hanahan and Weinberg and its later update (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011), there are six biological capabilities acquired during the process of carcinogenesis, which together support a multistep development of human tumors. As their maybe most fundamental property, cancer cells show the capability to sustain chronic proliferation. That is, while in normal cells cell growth and division are tightly regulated through growth-promoting cues primarily derived from the cell environment, cancer cells get decoupled from the control by these signals and "become masters of their own destinies" (Hanahan and Weinberg, 2011). For this purpose, cancer cells may produce growth factors by themselves or stimulate neighboring cells to do so, they may increase receptors for these factors or structurally alter these receptors rendering them hyper-response or become independent from external factors by constitutive activation of the pathways normally stimulated. In addition, negative feedback signals that normally reduce cell growth under such conditions can be disrupted and signals inducing senescence or apoptosis are disabled. A second hallmark is the evasion from growth suppressors which normally become activated. This includes alterations of the two prototypical tumor suppressor proteins retino-blastoma protein Rb and p53, as well as overcoming mechanisms of contact inhibition, which normally restrict growth of cells to ascertain tissue homeostasis. A third acquirement favoring tumor development and progression is a resistance to cell death, overcoming another barrier naturally keeping cell numbers relatively constant. This resistance is achieved through inactivation of cell death proteins, overexpression of rescue proteins, or inhibition of signaling pathways sensing cell damage. In order to sustain cell growth chronically, cancer cells must undergo changes enabling replicative immortality, which is mainly associated with altered telomere length and telomerase activity, both of which limit cell replications in non-cancerous cells. Further essential stages of tumor development require angiogenic signaling by the cancer cells, securing nutrient and oxygen supply sufficient for continuing growth, and finally activation of invasion and metastasis, which is the least understood hallmark still being heavily explored. A scheme depicting the most important steps is shown in Figure 1.

The molecular basis of many of these changes are mutations of genes encoding for the proteins involved in these processes. These mutations may be either inherited, i.e. passed on from parent to child, or acquired during a person´s life. Most of the acquired mutations do not cause cancer but lead the affected and often damaged cell to undergo programmed cell death or be recognized by immune cells and thereby eliminated. However, some mutations affect genes involved in cell growth or cell death or, more generally, so called oncogenes, and it is believed that once several mutations act together cells may undergo rapid further mutations transforming a merely fast-growing population of cells into a tumor cell mass eventually aggressively spreading across the body and invading organs distant from the site of development.

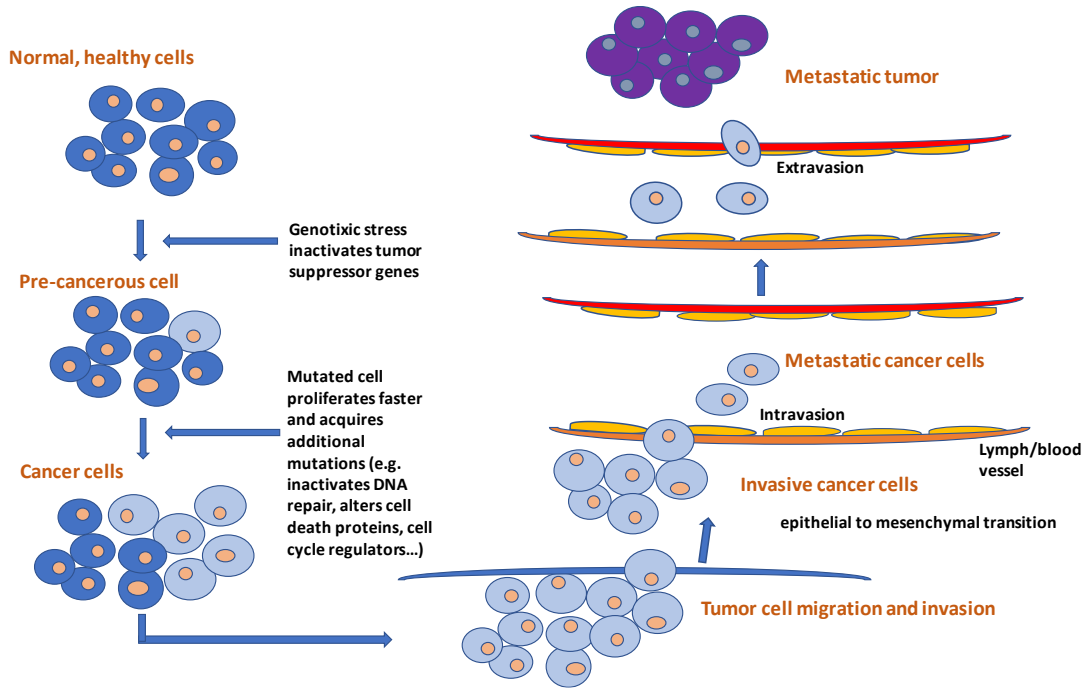

Figure 1. Cancer cell development and metastasis according to current concepts.

A number of genes have been found to be specifically related to breast cancer in that their mutation significantly enhances the risk of developing breast cancer, the most important of these genes being referred to as BRCA1 and BRCA2 (Breast Cancer gene 1 and 2). Both these otherwise unrelated proteins are involved in DNA repair and can in their mutated form no longer fulfill this function and hence favor the acquisition of further DNA mutations. Other genes, the expression of which is also by no means restricted to the breast, but which are significantly related with breast cancer development, are PTEN, a phosphatase involved in cell cycle regulation, the classical

tumor suppressor p53, CDH1, a member of the cell adhesion-related cadherin family of proteins, and serine/threonine kinase STK11, a regulator of cell polarity and growth (Shiovitz and Korde, 2015).

The specific role of estrogens in breast cancer development was described above. However, besides mutations affecting estrogen metabolism and signaling and indirect effects exerted by metabolites, it is presumably mainly the growth stimulatory action of the hormone which can make estrogen a dangerous molecule in the context of many of the above-mentioned mutations favoring cell growth and survival. Accordingly, as outlined before, the therapeutic treatment of ER-positive tumors usually involves hormone blocking therapy and the exposure to exogenous estrogens is in consequence prohibited during cancer treatment (Holmberg et al., 2008; Senkus et al., 2015). However, this conclusion is not unequivocally supported by the scientific community and is specifically debated among physicians applying the modern Western medical approach and combining it with TCM. Since some of the treatment strategies employed by TCM to treat breast cancer patients involve the use of substances with estrogenic action (Huang and Yu, 2016; Yuan et al., 2012), it appears of interest to have a closer look into this topic.

VIII. Types of estrogens

Although, in the chapters above the term estrogen was largely treated as if representing a single molecule and to some extent also related metabolic intermediates, there is actually a considerable number of diverse compounds referred to as estrogen-like substances. In humans, the principle endogenous molecules with estrogenic activity are estrone, estradiol and estriol, the relative abundance of which vary according to the reproductive stage of a female. In addition, there are three main types of estrogens not produced within the human body. As such, these are all subsumed as xenoestrogens by some. However, a better distinction which is more widely applied is grouping them according to their origin, discerning estrogens that are either produced by plants, derived from chemicals present in our environment, or which have been synthetically produced on purpose (Table 4).

Table 4: Types of estrogens not produced in the human body and examples

Group	Example	Source
Phytoestrogens		
Isoflavones	Genistein, daidzein	Soy beans, fava beans
Polyphenols	Resveratrol	Grapefruit, red wine
Catechins	Proanthocyanides	Chocolate, green tea
Xenoestrogens		
Bisphenols	Bisphenol A	Plastic production
Cyanuric chloride	Atrazin	Herbicide
Organochlorine	Endosulfan	Insecticide
Mycoestrogens	Zearalenone	Fusarum sp.
Synthetic estrogens		
Ethinylestradiol		Birth control pill
Estradiol valerate		Hormone replacement therapy

Plant-derived estrogenic substances are referred to as phytoestrogens. Typically, plant-derived estrogens have much weaker estrogenic activity than estrogens produced within the body and in some cases, they even have anti-estrogenic effects. Many of the phytoestrogens can be found in foods, the single most important source being soy, which contains isoflavones. Other examples are polyphenols, such as the well-known Resveratrol contained in grapefruit and red wine, or catechins contained in chocolate or green tea.

Xenoestrogens originate from environmental chemicals and many of these derive from petrochemicals. Just as phytoestrogens, they may act stimulatory or inhibitory on ER, and some act much more potently than the endogenous molecule. Together with several other hormone-like acting substances, these molecules are referred to as endocrine disrupters. Given the high degree of conservation of estrogens and ER across the animal kingdom, xenoestrogens do not only affect humans but exert profound effects on many animals, particularly those living in the water and being exposed to xenoestrogens

released from human settlements with the wastewater (Williams et al., 1998). However, also humans are being increasingly exposed to xenoestrogens through environmental pollution, and many xenoestrogens are clearly linked with enhanced cancer incidence in the reproductive system, breast, lung, kidney, pancreas, and brain (Fucic et al., 2012). A very prominent xenoestrogen representative is Bisphenol A, a compound used for the synthesis of plastics, which has been heavily released into the environment over the past decades. Despite the fact that it is not considered toxic, it is strongly suspected to cause or at least enhance the risk of developing breast cancer (Shafei et al., 2018; Yang et al., 2009). Other examples of xenoestrogens are the widely used herbicide Atrazin (meanwhile banned in the E.U.), and Endosulfan, a widely applied insecticide. Another group belonging to the xenoestrogens are the mycoestrogens, fungi-derived substances which are often found in stored grain and may exert considerable toxicity on livestock and poultry (Pereyra et al., 2008). Nonetheless, derivatives of the mycoestrogen zearalenone have been proposed for use in hormonal replacement therapy in postmenopausal women and as oral contraceptives (Pazaiti et al., 2012).

The third group of non-human estrogens are the synthetic estrogens. Whereas in some xenoestrogens which are also synthesized the estrogenic activity is an unwanted side effect, these are specifically produced by the pharmaceutical industry in order to act as hormone-like substances. Their most prominent use is for medications applied in birth control pills and for hormone replacement therapy, examples being ethinylestradiol and estradiol valerate, respectively.

IX. Chinese herbal medicine and cancer

In Western medicine, medicinal herbs have played an important role over a long period, but with the advent of pharmaceutical synthetic chemistry during the last century, their relevance has transiently lost considerably. Recently, however, interest was revived with an increasing recognition of the potential held in more comprehensive medical approaches including TCM. In contrast, in Eastern medicine TCM medicinal herbs have been continuously highly regarded and in the course of the recent modernization of Chinese medicine many traditional herbs have been preserved as medicine complementing the Western mechanism-centered approach and considerable efforts

have been undertaken to identify the mechanistic basis of their effects to enable their use in a Western pharmacological style (Efferth et al., 2007).

Besides their use in the therapy of many diseases including cancers, TCM herbs and preparations have traditionally been considered an important protective measure against the development of diseases (Kim, 2008; Messina et al., 2002; Patisaul and Jefferson, 2010), and an important constituent of many of these preparations are estrogenic substances (Huang and Yu, 2016; Kiyama, 2017; Yuan et al., 2012). A general overview on medicine applied in TCM exerting estrogen-like hormonal action has recently been provided by Wiebrecht (Wiebrecht, 2018) and a version complemented from additional sources is shown in Table 5. In line with this, the high proportion of soybean products in traditional Asian diets has been considered to underly the observation that Asian populations show historically lower rates of multiple diseases including cardiovascular diseases, diabetes, obesity, and also menopausal symptoms and breast cancer (Adlercreutz, 2002; Adlercreutz and Mazur, 1997). Given the role estrogens may play specifically in the latter diseases, it was assumed that among the constituents of soy products it was the phytoestrogens that would mainly account for these effects. In consequence, soy products were considered as healthy and became increasingly popular in the Western world both for the treatment of, e.g., menopausal problems (Murkies et al., 1995) and also as food ingredients in the health food sector (Brouns, 2002). In addition, due to the high protein content of soy beans, soy proteins found their way into multiple products serving as a cholesterol-free, non-animal derived substitute for animal products (Patisaul and Jefferson, 2010).

Starting about 50 years ago, this led to a broad discussion on the benefits and, with some delay, potential risks of food containing phytoestrogens and in the course of these 50 years many studies were undertaken evaluating whether uptake of phytoestrogen-containing food helps preventing diseases and reducing cancer risk and if treatment of cancer patients with phytoestrogen-containing medicine in addition to standard Western therapy is beneficial or, rather contrary, may even enhance the risk for reoccurrence of cancer. These considerations were also shared by Chinese scientists, who are in general supportive regarding the use of TCM medicine (Huang and Yu, 2016; Yuan et al., 2012).

Table 5: Herbs used in TCM with known estrogen-like effects. Modified after Wiebrecht 2018, Huang and Yu, 2016, and Yuan et al. 2012.

TCM medicine (scientific name)	Pinyin (transliteration of the Chinese ideogram)	Therapeutic effect attributed by TCM
Achillea millefolium herba	Yángshīcǎo	Humor venti diverting
Achyranthis radix (= Cyathulae radix)	Niúxī	Xue dynamyzsing
Agrimoniae herba	Xiānhècǎo	Xue preserving
Alismatis rhizoma	Zéxiè	Humor diverting
Angelicae sinensis radix	Dāngguī	Xue preserving and supplementing
Artemisiae absinthii herba	Kǔ'ài	Calor reducing
Astragali radix	Huángqī	Qi supplementing
Atractylodis macrocephalar rhiz.	Báizhú	Qi supplementing
Belamcandae rhizoma	Shègān	Calor reducing and desinfecting
Brassica oleracea	Gǎnlǎn	Digestive
Bupleuri radix	Cháihù	Extima opening and cooling
Carthami flos	Hónghuā	Xue regulating and dynamizing
Cassiae semen	Juémíngzǐ	O. hepaticus cooling and ventus diverting
Chuanxiong rhizoma	Chuānxiōng	Xue dynamizing
Cervi cornu pantotrichum	Lùróng	Yang supporting and supplementing
Cicer arietinum	Huíhuídòu	Calor reducing
Cinnamomi cassiae cortex	Ròuguì	Intima warming
Cinnamomi cassiae ramulus	Guìzhī	Extima opening; humor venti diverting
Cistanchis herba	Ròucōngróng	Yang supporting and

		supplementing
Cnidii fructus	Shéchuángzǐ	Yang supporting and supplementing
Codonopsitis radix	Dǎngshēn	Qi supplementing
Coicis semen	Yìyǐrén	Humor diverting
Cordyceps	Dōngchōngxiàcǎo	Yang supporting and supplementing
Curculiginis rhizhoma	Xiānmáo	Yang supporting and supplementing
Curcumae radix	Yùjīn	Xue regulating and dynamizing
Cuscutae semen	Tùsīzǐ	Yang supporting and supplementing
Cyathulae radix (= Achyranthis radix)	Chuānniúxī	Xue dynamizing
Dalbergiae odoriferae lignum	Jiàngxiāng	Xue regulating and dynamizing
Dioscoreae nipponicae rhizoma	Chuānshānlóng	Humor venti diverting
Dioscoreae rhizoma	Shānyào	Qi supplementing
Drynariae rhizoma	Gǔsuìbǔ	Yang supporting and supplementing; Xue regulating and dynamizing; humor venti diverting
Ecliptae herba	Hànliáncǎo	Yin supporting
Epimedii herba	Yínyánghuò	Yang supporting and supplementing
Eucommiae cortex	Dùzhòng	Yang supporting and supplementing
Flemingiae philippensis radix	Qiānjīnbá	Humor venti diverting
Foeniculi fructus	Xiǎohuíxiāng	Intima warming
Fallopia convolvulus herba	Juǎnjīngliǎo	Digestive

Ganoderma	Língzhī	Shen supporting
Ginseng radix	Rénshèn	Qi supplementing
Ginseng roter	Hóngshèn	Qi supplementing
Glycine max	Huángdàdòu	Digestive
Glycyrrhizae radix	Gāncǎo	Qi supplementing
Granati pericarpium	Shíliúpí	Astringent
Hibisci sabdariffae calyx	Méiguīqié	Astringent
Jujubae fructus	Dàzǎo	Qi supplementing
Kaempferia rhizoma	Shānnài	Intima warming
Ligustri lucidi fructus	Nǚzhēnzǐ	Yin supporting
Lupuli flos	Píjiǔhuā	Digestive
Lycii fructus	Gǒuqǐzǐ	Yin supporting
Moutan cortex	Mǔdānpí	Xue cooling
Medicago	Mùxù	Calor reducing
Myristicae semen	Ròudòukòu	Intima warming
Ocimi basilici herba	Luólè	Extima opening
Paeoniae radix lactiflorae	Báisháo	Xue preserving and supplementing
Paeoniae radix rubrae	Chìsháoyào	Xue cooling
Phaseolus radiatus	Lǜdòuyá	Calor reducing
Phaseolus vulgaris	Càidòu	Depletio supplementing
Plantago major	Dàchēqián	Humor diverting
Polygoni cuspidati rhizoma	Hǔzhàng	Xue regulating and dynamizing
Polygoni multiflori radix	Héshǒuwū	Xue preserving and supplementing
Psoraleae semen	Bǔgǔzhī	Yang supporting and supplementing
Puerariae radix	Gégēn	Extima opening and

		cooling
Rehmanniae radix	Shēngdìhuáng	Xue cooling
Rehmanniae radix praeparata	Shúdìhuáng	Xue preserving and supplementing
Salviae miltiorrhizae radix	Dānshēn	Xue regulating and dynamizing
Schisandrae fructus	Wǔwèizǐ	Astringent
Selaginellae herba	Zhōnghuájuǎnbǎi	Calor reducing
Selaginellae moellendorfii herba	Dìbǎizhī	Extima opening
Selaginellae tamariscinae herba	Juǎnbǎi	Xue dynamimng
Sophorae tonkonensis radix	Shāndòugēn	Calor reducing and desinfecting
Trifolii pratensis herba	Hóngchēzhóucǎo	Calor reducing
Vicia faba	Cándòu	Humor diverting
Zingiberis rhizoma	Gānjiāng	Intima warming
Ziziphi spinosae semen	Suānzǎorén	Shen supporting

Similarly, in a recent discussion of this issue, Wiebrecht (Wiebrecht, 2018) pointed out the potential risk of estrogenic substances in the context of breast cancer, but considered them overall beneficial rather than detrimental. However, most of the relevant investigations were either retrospective or less often prospective epidemiological studies, and only few have addressed mechanistic aspects, elucidating the impact of phytoestrogens on cellular processes. However, various studies have previously hinted at a potential interference of phytoestrogen with cancer therapeutic drug action (Goodson et al., 2011; Ju et al., 2008), and recently Warth and colleagues provided evidence that phytoestrogens may potently inhibit the action of a novel combination-therapy against ER-positive breast cancer, suggesting that phytoestrogen-containing substances should be strictly avoided by breast cancer patients (Warth et al., 2018). The subject is thus clearly not solved and there is quite contradicting evidence in support for either side. Therefore, in the following an overview will be given on some exemplary

studies and meta-analyses published in this context, including the most recent studies that could not be considered in previous reviews on this subject (Adlercreutz, 2002; Patisaul and Jefferson, 2010; Rice and Whitehead, 2006).

X. Estrogens in nutrition and herbal treatments and their impact on breast cancer

Exemplary studies showing positive effects of estrogenic substances

Lee et al., Lancet 1991

An early study investigating the role of dietary intake of 90 foodstuffs, differentiating among nutrients, animal or plant source etc., investigated 200 Singapore women with breast cancer and 420 healthy patients using a quantitative food -frequency questionnaire. With this questionnaire, food habits one year prior to the interview and, in cancer patients, 1 year prior to diagnosis were collected. Additional assessed parameters included menstrual and child bearing history, oral contraceptive use and breast feeding, and also family history of breast disease, smoking habits, height and weight.

Using multiple logistic regression analysis, odds ratios and deviance chi-squared tests of effects were calculated, differentiating between pre- and post-menopausal women.

Adjusting for age and age at first birth, the analysis showed that in pre-menopausal women an increased breast cancer risk was associated with a high relative intake of animal protein and red meat, whereas a decreased risk was correlated with high intakes from polyunsaturated fatty acids, beta-carotene, soy proteins, and total soy products. In comparison, in post-menopausal women, none of these relations were statistically significant. Various other adjustments did not affect these results, suggesting that dietary impacts were restricted to pre-menopausal women. Not surprisingly, this led the authors to suggest that a main decisive factor on the dietary impact on the risk of breast cancer development is the hormonal background of the women examined. In line, the authors also correlated the enhanced incidence of breast cancer in Singapore observed in the periods preceding to the study with a change in dietary habits, which would also

affect younger women more than older ones, the latter clearly making up a larger share of post-menopausal women. Further, the authors speculate that the apparent protective effect of soy beans could be related to phytoestrogens contained in these, suggesting that they may act to suppress endogenous estrogenic activity and thereby inhibit hormone-dependent carcinogenesis.

Trock et al., J. Natl. Cancer Inst. 2006

Numerous investigations were performed following the insights and suggested implications reported by Lee and colleagues and related studies to address the interplay between phytoestrogen uptake in the food and the risk of breast cancer development. In consequence, Trock et al. were able to provide a meta-analysis in 2006 covering a total of 18 studies conducted between 1978 and 2004 examining soy intake and breast cancer risk and comprising a total of approximately 20.000 women. It was noted by the authors that the control for confounding factors varied quite a lot between studies and thus numerous assumptions had to be made. E.g. when tofu intake was converted to soy protein intake, differences between relative soy content in Western and Asian tofu had to be accounted for, and in two studies where urinary isoflavone secretion was measured the conversion to soy protein taken up had to be estimated. When pooling the relative risk estimates derived from these studies and accounting for determined or estimated soy protein intake, it was seen that high soy intake modestly correlated with a reduction in breast cancer risk, the odds ratio being 0.86. Accounting for menopausal status, where it was reported, it was noted that there was a stronger impact observed in pre-menopausal women (odds ratio 0.70) than in post-menopausal women (odds ratio 0.77). The authors mentioned that in many of the individual studies no significant correlation was observed and that e.g. in 6 out of 8 studies that failed to report the menopausal status, soy protein uptake and breast cancer risk were unrelated. It was further reported that the beneficial effect was slightly more pronounced in Women from Western countries (odds ratio 0.84) as compared to Asian women (0.89), but when correcting for Asian Americans included in the former studies this difference vanished.

In their interpretation of the data, Trock and colleagues appreciate the many uncertainties associated with their overall finding. For example, the fact that the reduction by high soy protein intake was comparable in Western and Asian countries

seems odd when in fact the total intake by Western women was much lower than that of Asian women. They suggest that high soy intake by Western women may in fact reflect a generally more health-conscious life style and thus be a surrogate for other risk-lowering factors. On the other hand, they also note that breast cancer risk in Western women is much higher than in Asian women, 133 per 100.000 and 39 per 100.000, respectively, but this may as well relate to multiple other risk factors such as late age at first full-term pregnancy, early menarche, obesity, alcohol and other adverse nutritional factors.

Verheus et al., J. Clin. Oncol. 2007

An investigation aimed at establishing a more direct link between phytoestrogen-exposure and breast cancer risk was published by Verheus et al. in 2007. Different from the beforementioned studies it did not evaluate soy protein intake or a similar surrogate for phytoestrogen exposure, but measured plasma levels of estrogen-like substances derived from plant sources, comprising isoflavones daidzein, genistein and glycitein and the lignans enterodiol and enterolactone. The latter represent phytoestrogens primarily taken up through cereals, flaxseed and berries. Participants of the prospective study were 383 patients and controls, sampling being conducted before diagnosis and assessment of lifestyle factors and dietary habits.

The study showed that for isoflavones an association between high plasma levels and lowered breast cancer risk could be established. The strongest impact was detected for genistein, displaying an odds ratio of 0.68, i.e. a 32% reduction in breast cancer risk comparing the upper tertile of plasma levels with the lowest tertile. The relation held for both pre- and post-menopausal women, but was less stable for the former, presumably due to a limited case number. None of these relations was significantly affected by adjustment of any of the confounding factors.

For the lignans, no relationship between their plasma levels and breast cancer of the entire population was observed. In contrast, in pre- and peri-menopausal women high plasma levels of enterolactone appeared to increase rather than decrease breast cancer risk (odds ratio 1.71), but this was not significant.

Overall the authors conclude that high plasma genistein levels are associated with a lowered breast cancer risk in a primarily white Western population, despite of low

overall circulating levels of isoflavones. They suggest that other isoflavones may have similar effects but consider the evidence not strong enough to claim this.

Cui et al, Am. J. Epidemiol. 2006, and Lee et al., Cancer 2014

Of the rather few studies directly addressing the impact of TCM medicine on breast cancer two shall be briefly discussed here. In the epidemiological study by Cui and colleagues, 1455 breast cancer patients recruited in a Shanghai Brest Cancer Study were investigated with regard to the impact of their ginseng use before and post diagnosis and their overall and disease-free survival and their quality of life. Ginseng contains saponins, which are known to exert phytoestrogen-related effects. It was observed that about 27% of the patients were regular ginseng users and these had a significantly reduced risk of death. The adjusted hazard ratios associated with ginseng use were 0.71 for total mortality and 0.70 for disease-specific mortality. It was also noted that ginseng use after diagnosis was associated with elevated scores of quality of life, particularly in the psychological and social well-being domains.

In the study by Lee et al. 729 patients with advanced breast cancer receiving taxanes were analyzed in a retrospective population-based cohort study. Patients were divided in TCM users (115 patients) and TCM nonusers (614 patients) and the death reports during a mean follow-up time of 2.8 years and observed during a 10-year overall period were evaluated. Multivariate analyses indicated that TCM users had a significantly reduced risk of all-cause mortality, the adjusted hazard ratio to 0.55 for patients using TCM for 30 to 180 days, and to 0.454 for patients using TCM longer than 180 days. The most effective TCMs in terms of reducing mortality were reportedly Bái Huā Shé Cǎo, Bàn Zhī Lián, and Huáng Qí.

While this seems to strongly support the use of TCM in breast cancer patients, there are several limitations of the study, as pointed out by the authors themselves. E.g., the number of patients observed was rather limited, it could not be assessed how compliant the TCM users were with their prescriptions, and, most importantly, it was not possible to analyze the breast cancer related mortality. Thus, this study is clearly only observational, and its focus on women with advanced breast cancer treated with taxenes prohibits any generalization of the results. Additionally, it should be considered that such more or less positive effects of TCM drugs in a situation of ultimately presumed

fatal outcome, as is typical for the metastasized situation, is not comparable with the adjuvant situation where each risk factor for disease recurrence has to be strictly avoided.

Studies on ethnicity related effects

Some of the studies conducted over the years seemed to suggest that there is a population difference regarding the impact of phytoestrogens. Shu et al. (Shu et al., 2009) and Zhang et al. (Zhang et al., 2010) clearly confirmed the beneficial effect of high soy food consumption in terms of a reduction of breast cancer risk in Chinese populations and Nechuta et al. (Nechuta et al., 2012) showed that in breast cancer survivors post-diagnosis survival was enhanced and breast cancer recurrence reduced in a 7.4 year follow-up both in Chinese and US American patients. Similarly, a comparative study on Japanese-American and white women detected a decreased breast cancer risk in both populations correlating with urinary phytoestrogens excretion (Goodman et al., 2009). In contrast, a number of other studies (Chen et al., 2014; Dong and Qin, 2011; Wu et al., 2008; Xie et al., 2013) corroborated a protective effect in Asian populations but saw no effect in Western populations.

Among the reasons suggested to underly this difference is that in Asian countries soy consumption by far exceeds that in Western populations, both expressed as daily intake and as that over life time. In addition, Western life style involves numerous other risk factors enhancing breast cancer risk related to sexual development, bearing and breast-feeding children, obesity, alcohol and smoking.

However, a recent study investigating the impact on isoflavone intake on survival in breast cancer patients from multiple ethnic groups registered in Canada and the USA could not detect any differences between non-Hispanic whites, Hispanics, blacks, Asians and others (Zhang et al., 2017). Thus, independent of ethnicity women from all these groups showed a reduced all-cause mortality when dietary isoflavone was high as compared to low. Noteworthy, Asians did not differ in this regard despite of the fact that they consumed considerably more isoflavones than other groups (6.1 vs 1.3 mg daily) but still substantially less than women living in Asian countries (e.g. 45.9 mg daily in China). Furthermore, it appears particularly interesting that this effect was only significant for women diagnosed with ER/PR-negative tumors and women who did not

receive hormone-therapy. At the same time, the study could not detect any detrimental effect of high isoflavone intake on survival in women undergoing hormone-therapy. Another interesting facet of the study was that the beneficial effect was only significant for post-diagnosis isoflavone intake, whereas the relationship was much smaller with pre-diagnosis intake, suggesting that the more recent diet is more important than a more remote one.

Another recent study addressed the hypothetical ethnicity-related differences with a different approach, examining the levels of various breast cancer risk factors in Asian and non-Asian pre-menopausal women involved in a 2-year intervention study (Maskarinec et al., 2017). For this purpose, women were assigned to either a high or a low soy protein diet and at the end of the study period numerous typical markers for breast cancer and for inflammation were examined. As it turned out, ethnic differences in the levels of some biomarkers for breast cancer risk (although mostly not statistically significant) were indeed identified (e.g. serum C-reactive protein, serum leptin, nipple aspirate fluid volume, nipple aspirate fluidestrone sulfate, urinary isoflavones, IGF-1), but at the same time it appeared that Asian women did not respond differently to soy foods than non-Asian women.

Table 6: Studies supporting beneficial effects of phytoestrogens in the prevention or treatment of breast cancer

Study subjects	Effect	Reference
Chinese (Singapore) population (200 patients vs 420 controls	Protective effect of soybean in diet	Lee et al., 1991
Meta-analysis on 18 studies in Western and Asian countries	Protective effect of soybean in diet	Trock et al., 2006
Chinese population (1455 breast cancer patients)	Ginseng use enhanced survival and quality of life	Cui et al., 2006
European (Dutch) population (383 patients vs 383 controls)	Genistein reduced breast cancer risk; lignan had no effect	Verheus et al., 2008
Multi-ethnic US Americans, Japanese Americans and Latin Americans (36.485 post-	High urinary excretion of phytoestrogens correlates with lower breast cancer risk in post-	Goodman et al., 2009

menopausal patients)	menopausal women	
Asian and Western populations from 18 studies	Isoflavone consumption was inversely related with breast cancer risk in Asian but not Western populations	Dong et al., 2011
US American and Chinese breast cancer survivors (9.514 patients)	High post-diagnosis soy food consumption enhanced survival and reduced recurrence	Nechuta et al., 2012
Multi-ethnic American breast cancer patients (6.235 patients)	High isoflavone intake reduced all-cause mortality in women with ER-negative tumors, independent of ethnicity	Zhang et al., 2017
Meta-analysis on 22 studies in Western and Asian countries	Exposure to high isoflavone reduced risk of breast cancer in Asian but not Western populations and in pre- but not post-menopausal women	Xie et al., 2013
Taiwanese breast cancer patients (729 patients with advanced breast cancer)	Adjunctive TCM therapy may lower the death rates in patients with advanced breast cancer	Lee et al., 2014

In summary, multiple studies and meta-studies observed that a high intake of isoflavones can be linked with a certain reduction of breast cancer risk. In addition, some studies found that high intake of isoflavones is also beneficial in breast cancer patients undergoing cancer therapy. However, several discrepancies occur when comparing these studies showing that the protective effect may or may not be limited to certain ethnical groups, that it can either be found in all or only in pre-menopausal or post-menopausal women, and that it can only be detected in ER-negative breast cancers. These results leave us with considerable uncertainties and in particular the later findings seem to put any mechanistic hypothesis derived from the other studies into question. A summary of the studies mentioned here which report a beneficial effect of phytoestrogens on breast cancer risk is provided in Table 6.

Exemplary studies showing no effects (epidemiologic and mechanistic)

From the studies discussed above one might argue that a high intake of isoflavones and other phytoestrogens should nonetheless be aspired to, since, if there is no benefit it might at least not hurt any patient. However, over the years an increasing number of studies produced results that indicate the opposite, i.e. they showed that phytoestrogens may not be neutral in the worst case, but actually increase breast cancer risk or interfere with therapeutic approaches against cancer. Exemplary studies documenting this and highlighting some mechanistic aspects shall be summarized in the following (Table 7).

Key et al., Br. J. Cancer 1999, Keinan-Boker et al. Am. J. Clin. Nutr 2004, and Peeters et al. Breast Cancer Res. Treat 2003

An exemplary investigation showing no effect of dietary soy food intake on breast cancer risk is that by Key and colleagues (Key et al., 1999). In this prospective study, a total of more than 34.000 women from Hiroshima and Nagasaki completed dietary questionnaires and were followed for incident breast cancer for up to20 years. The data were acquired in the course of a larger study evaluating, among other facets, the impact of irradiation due to the atomic bombings of both cities and thus the data were also stratified for absolute age, “age at the bomb”, and radiation exposure. Analyzing the data for the relative intake of tofu, miso soup and multiple other foods and drinks, none of these showed a significant relation with breast cancer risk. This was true for women below and above 50 years of age and it was apparently also independent from the radiation dose exposed to. The authors admit that there are certain weaknesses of the study, e.g. the fact that isoflavone contents of soy food could not be exactly quantified, but also state that their study confirms various others conducted on Japanese and Chinese women before.

In a study on more than 15.000 Dutch women between 49 and 70 years of age, the intake of isoflavones and lignans was also not found to be significantly related with breast cancer risk (Keinan-Boker et al., 2004). In a meta-analysis published by the same group, 18 studies of this type were examined, including 13 prospective studies, and from their overview the authors concluded that there is no protective effect to be

detected, but they appreciate that exceptions may exist for women who consume phytoestrogens at adolescence and for those who consume very high doses (Peeters et al., 2003), indicating that this needs further investigation.

Table 7: Studies indicating no impact of phytoestrogens on breast cancer risk or the treatment of breast cancer

Study subjects	Effect	Reference
Japanese population (34.759 women)	No protective effect of soy food	Key eta al., 1999
Dutch population (15.555 women)	No protective effect of dietary isoflavones and lignans	Keinan-Boker et al., 2004
Meta-analysis on 18 studies	No protective effect of dietary phytoestrogens	Peeters et al., 2003

Epidemiological studies detecting negative effects of phytoestrogens on breast cancer risk.

Relatively early after the suggestion of beneficial effects of a high intake of phytoestrogens, there were also reports on potential negative effects of high estrogen levels. An example is the study by Ekbom et al. (Ekbom et al., 1992) in which prenatal influences on subsequent breast cancer risk were evaluated, comparing 458 breast cancer patients with 1197 matched controls. For all these women estrogen levels were inferred from multiple correlating factors (including fetal growth and pre-eclampsia and eclampsia) and there was a significant relation between the presumed exposure to high levels of endogenous estrogens and the risk to develop breast cancer. Given that it is still not unequivocally clear if phytoestrogens exert estrogenic or anti-estrogenic function, it remains unresolved if they would aggravate or ameliorate the situation. Related animal studies reported contradictory results, showing that rat pups of mothers consuming genistein during gestation and lactation were protected against developing breast tumors (Fritz et al., 1998), while neonatal mice injected with genistein displayed altered mammary gland development including “abnormal ductal morphology and focal areas of “beaded” ducts lined with hyperplastic ductal epithelium” (Padilla-Banks et al.,

2006). Administration of a low dose of genistein, however, accelerated mammary gland development without ductal malformations, and this was supposed to even reduce cancer risk and indicated that genistein dose may be an important factor in determining cancer risk. Yet another study, examining the impact of genistein and zearalenone by injecting pregnant rat with these phytoestrogens, found that when their offspring were subsequently exposed to a cancerogenic chemical there was a dose-dependent increase of breast tumor incidence detectable with genistein, but not zearalenone (Hilakivi-Clarke et al., 1999), suggesting that at least some phytoestrogens enhance the susceptibility to breast cancer.

A more direct approach involving humans, comparable with epidemiological studies discussed above, is that by Grace and colleagues (Grace et al., 2004). In this study the levels of 7 phytoestrogens, daidzein, genistein, equol and others, was measured in urine and serum samples from breast cancer patients and healthy controls aged between 45 and 75 years. Remarkably, although dietary phytoestrogen levels were generally low, their serum levels were up to 600 times greater than endogenous estradiol levels measured in post-menopausal women. Stratifying data for confounding variables, statistical analysis found that for all isoflavones there was an increased breast cancer risk associated with increased serum and/or urine levels, which reached significance for daidzein and equol. The study also found dietary intake of daidzein and genistein positively correlated with breast cancer risk, but this was not significant. Thus, although the study is based on a relatively small number of only 333 women examined, the fact that urine and serum levels of phytoestrogens were directly measured rather than inferred from food intake underlines its importance.

Finally, a 2-year intervention study needs mentioning, in which the interplay between soy food intake and mammographic densities were investigated (Maskarinec et al., 2004), the latter correlating with enhanced breast cancer risk. In the overall population of 220 pre-menopausal women examined, the authors did not find a significant impact of phytoestrogens on breast density, but in the Caucasian sub-population enhanced soy consumption did significantly correlate with breast density. Whether this ultimately translates into enhanced breast cancer risk, could of course not be answered in this study.

The most recent large-scale studies published on the impact of estrogen levels on breast cancer risk need to be mentioned, even though one of these did not evaluate

phytoestrogens as such. In this study covering 27.153 US-American women not only dietary intake, but several life-style related factors known to modify endogenous estrogen levels were evaluated and given an estrogen-related life-style score between 1 and 6, with a high score indicating a lower breast cancer risk and vice versa (Guinter et al., 2018b). The factors considered included estrogenic diet, alcohol intake, body mass index, and physical activity (Table 8).

Table 8: Factors considered for the calculation of an Estrogen-related lifestyle score. Compiled from Guinter et al., 2108 (Guinter et al., 2018b).

ERLS factor	Score	Description
ERDP = Estrogen-related dietary pattern;	0	≥ ERDP score
	1	< ERDP score
Alcohol use	0	Heavy: > 7 drinks/week
	1	Moderate: > 0–7 drinks/week
	2	Abstainer: 0 drinks/week
Weight status	0	Obese: BMI ≥ 30 kg/m2
	1	Overweight: BMI 25.0–29.9 kg/m2
	2	Normal weight: BMI < 25 kg/m2
Physical activity (PA)	0	Inactive: ≤ 2 h/week of vigorous PA
	1	Active: > 2 h/week of vigorous PA

ERDP was derived from the uptake of food with positively-weighted intakes (non-whole/refined grains, tomatoes, cruciferous vegetables, cheese, fish/shellfish high in ω-3 fatty acids, franks/luncheon meats) and of food with negatively weighted intakes (nuts/seeds, other vegetables, fish/shellfish low in ω-3 fatty acids, yogurt, coffee), assessed through a questionnaire.

The single most important finding was that in post-menopausal women the combined effects of a low estrogenic diet and a life-style favoring low endogenous estrogens were associated with a reduction in breast cancer risk. The same group separately analyzed dietary estrogen intake and derived a score for an estrogen-related dietary pattern. As

with the first study, the authors concluded that an increased risk was associated with increasing estrogen intake in post-menopausal women, although this was not significant (Guinter et al., 2018a). This suggests that accounting for dietary estrogen intake may not always be sufficient to obtain a good estimate for estrogen exposure, and this might also explain the lack of correlations observed in some previously published studies.

An overview of the studies mentioned here and reporting negative impacts of phytoestrogens on breast cancer risk is shown in Table 9.

Table 9: Studies indicating a negative impact of phytoestrogens on breast cancer risk or the treatment of breast cancer

Study subjects	Effect	Reference
Swedish population (458 breast cancer patients and 1197 controls)	Intrauterine exposure to high endogenous estrogens increases breast cancer risk	Ekbom et al., 1992
British population (114 breast cancer patients and 219 controls	High serum and urinary phytoestrogen levels correlate with enhanced breast cancer risk	Grace et al., 2004
Hawaiian population (220 women)	Enhanced soy intake increases breast density in Caucasian women	Maskarinec et al., 2004
US-American population (27.488 women)	Dietary pattern of enhanced estrogen intake is associated with enhanced breast cancer risk in post-menopausal women	Guinter et al., 2018a

Mechanistic studies indicating detrimental effects of phytoestrogens on breast cancer treatment

Overall, the number of epidemiological and intervention studies supporting a positive effect of phytoestrogens regarding breast cancer risk and/or therapy clearly exceeds those indicating a detrimental impact. However, numerous studies investigating the *in vitro* effects and the mechanisms of action of phytoestrogens, as well as several animal

studies, produced alarming results suggesting that phytoestrogens should be largely avoided or at least only applied under very controlled conditions.

The maybe best studied phytoestrogen in this regard is genistein, a phytoestrogen contained in soy and fava beans, red clover, many other plants, and part of numerous TCM medications (Huang and Yu, 2016; Yuan et al., 2012). This compound is known to inhibit several pathways important for cell growth and proliferation, including protein tyrosine kinases, and was thus supposed to slow tumorigenesis (Agarwal, 2000). In addition, it can inhibit DNA replication enzymes (Okura et al., 1988), and reduce the expression of various growth factors, including vascular endothelial growth factor (Ravindranath et al., 2004), which is important for tumor vascularization. A growth inhibitory effect of genistein was documented for a multitude of cells and in many cases this was due to induction of cell death (Taylor et al., 2009). A growth inhibition could even be documented for ER-positive MCF-7 breast cancer cells (Wang et al., 1996). However, paradoxically this inhibitory effect could only be detected at relatively low concentrations, where it was shown to occur via the ER pathway. In contrast, at higher concentrations genistein stimulated growth through an ER-independent mechanism. This observation was substantiated in a mouse model treated to harbor sub-cutaneous estrogen-dependent tumors (Allred et al., 2001). When these mice were fed soy protein isolates, this induced a dose-dependent stimulation of tumor growth and the concurrent activation of estrogen-responsive genes suggested that this occurred through the ER pathway.

In a study using genistein and apigenin, another phytoestrogen, this growth stimulatory action was confirmed for ER-positive MCF-7 and ER-alpha-positive T47D cells, another human breast cancer cell line (Seo et al., 2006). Importantly, in addition this study also reported that genistein antagonized the growth-inhibitory action of the anti-estrogenic drug hydroxytamoxifen, the most commonly used drug for the treatment of ER-positive breast cancer both in pre- and post-menopausal women (Shagufta and Ahmad, 2018). This observation leads us to the consideration of a final series of publications, documenting the interference of phytoestrogens with therapeutic treatments applied to breast cancer patients.

For example, in another mouse implanted breast cancer model the impact of genistein on the breast cancer therapeutic effect of the aromatase inhibitor letrazole was tested (Ju et al., 2008). For this purpose, mice received silastic implants containing letrazole and

the aromatase substrate androstenedione and where then injected with aromatase-expressing MCF-7 cells. The expression of aromatase is typically found in breast tumors, where it is upregulated up to 100-fold and serves to produce endogenous estrogen to support tumor growth. Then mice were fed with variable doses of genistein and tumor growth was monitored over up to 19 weeks. It turned out that genistein dose-dependently inhibited tumor growth, when the aromatase substrate was not present in the mice. In comparison, the androstenedione-positive mice produced the maximum tumor growth among all groups and this tumor growth was fully inhibited in the presence of the aromatase-inhibitor letrazole, while this effect was again dose-dependently offset in the concurrent presence of genistein. The authors thus conclude that the dietary intake of genistein by women with ER-positive breast cancer bears a considerable risk as it may negate the beneficial effects of letrazole treatment. This was functionally confirmed in an *in vitro* breast cancer model, where genistein negated the growth inhibitory action of another aromatase inhibitor, fadrozole, at physiologically relevant concentrations (van Duursen et al., 2011).

Finally, an important study shall be briefly discussed which actually initiated the interest in the current topic, an investigation by Warth et al. (Warth et al., 2018). In this study, the impact of genistein and zearalenone on growth inhibition exerted on MDF-7 and T47F breast cancer cells by the drug combination palbociclib and letrozole was investigated, representing a DCK-4/6 inhibitor and an aromatase inhibitor, respectively. After confirming the strongly synergistic effect of both cancer drugs, making this an enormously efficient novel first-line treatment for advanced ER-positive breast cancer (Wolff, 2016), the effect of the phytoestrogens was evaluated. The experiments revealed that both of them significantly inhibited the growth-retarding effect of the cancer drugs and shifted the metabolomic pattern displayed by the cells to one indicating that the anti-oncogenic effects of the drugs were circumvented. Accordingly, the authors conclude that the intake of bioactive food chemicals, such as phytoestrogens, may counteract the metabolic and anti-proliferative effect of the drug combination also *in vivo* and thus they consider an impact on the therapeutic success plausible.

XI. Recommendations derived from this comparison

Although the current work accounts for only some, but clearly not all the studies conducted over the past decades to elucidate the role of estrogens in breast cancer, it covers the most recent and the most important works on the subject. From this overview it should have become clear that this subject has been intensively studied. Nonetheless, there is still no clear picture that can be drawn from these investigations and it remains controversial whether phytoestrogens act in a positive or negative manner. Thus, even in epidemiologic studies comprising data from thousands of women almost any study supporting beneficial effects of phytoestrogens can be opposed with another study showing either no effect or even negative ones. Importantly, even positive studies may contradict each other in that in one the effect may be restricted to pre-menopausal women, while in the other it is found true only for post-menopausal women. Some of these discrepancies may be related to uncertainties regarding the quantification of phytoestrogen intake which is particularly difficult when examining women living in different regions of the world where e.g. soy products were shown to contain quite different levels of phytoestrogens (Trock et al., 2006). However, while one might be led to suspect that this could then underlie the fact that high estrogen intake could be related with ethnicity, showing protective effects in Asian but not Caucasian populations (see studies discussed above), studies on different ethnical groups inhabiting the same regions could not confirm such differences. It thus appears more likely that simply accounting for dietary estrogen intake is not an adequate measure to estimate overall estrogen exposure and that other factors need to be considered as well, as shown by Guinter and colleagues (Guinter et al., 2018a; Guinter et al., 2018b).

Few studies have directly addressed the impact of TCM medicines, but some of these, as exemplified in the two studies briefly discussed here (Cui et al., 2006; Lee et al., 2014), suggest positive effects on survival of breast cancer patients. The fact that these studies were limited to advanced breast cancer patients prohibits any generalization, but as specifically the study by Cui et al. reported an enhanced quality of life score in the patients, it appears that the use of TCM medicines for adjunctive treatment may indeed be beneficial under some conditions. The decision whether to use TCM medicine in this

specific situation or not will need careful consideration on a case-to-case basis and the overall picture obtained here will in case of doubt rather discourage from its use.

One factor that may be particularly important in this context is the application of drugs containing phytoestrogens. The exact levels and the nature and composition of estrogenic substances may not be known in Chinese preparations designed to treat e.g. menopausal problems, but many constituents of these are known to contain estrogenic substances and have presumably been selected to contain these (Yuan et al., 2012). Applying such drugs or drug-derived additives for the treatment of menopausal problems has been documented to be truly beneficial (Kargozar et al., 2017; Yuan et al., 2012) and one might argue that in the absence of opposite findings the use of such drugs for otherwise healthy patients is safe. However, in the light of the above mentioned *in vitro* and animal studies, this does not seem true for breast cancer patients that have been treated previously or are still undergoing therapy. As documented by the studies discussed above, there exists a considerable risk that phytoestrogens may interact with and effectively negate the anti-cancer effect of therapeutic drugs and thus exert detrimental pro-cancer action. Somewhat surprisingly, this may not even be restricted to patients with ER-positive breast cancer, since some of the *in vitro* effects appeared to be mediated through ER-independent pathways, the nature of which remains to be detected. As a cautionary recommendation it may thus be concluded that in breast cancer patients the use of phytoestrogen-containing drugs shall be avoided as it is not safe. A potential problem associated with this recommendation is that estrogenic substances may not be clearly identified for many TCM drugs currently used. At present there are no simple detection methods for estrogenic substances available and those tests that are available at all are either infrastructure- and time-intensive (e.g. detection of a proliferative effect on human breast cancer cells in culture over 1-2 weeks) or require previous knowledge of the chemical structure of the expected compound (HPLC-based methods). Thus, an easily applicable, fast and reliable method for the detection of estrogenic substances is clearly needed to allow the practicing physician to act according to the above-named recommendation.

At present, numerous standard therapies for the treatment of breast cancer are well established while a concurrent patient-specific approach towards personalized medicine is become increasingly realistic. Importantly, the decision on which therapy to apply does generally not rest on the shoulders of a single responsible physician but is decided

through a tumor board review by a team of physicians with expertise in different medical fields. Thus, at present even the use of TCM medicine may be decided upon discussion among various experts and may in consequence be tailored to fit each individual case. As long as truly conclusive studies on the safety of the use of phytoestrogen-containing TCM medicines are still missing, the practicing physician not specialized in breast cancer treatment should rather rely on these conventional approaches and refrain from the use of drugs exerting unknown effects.

XII. References

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich

- die vorliegende Masterarbeit selbständig und ohne unerlaubte Hilfe angefertigt habe,
- andere als die angegebenen Quellen und Hilfsmittel nicht benutzt habe,
- die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlichgemacht habe und
- die Arbeit in gleicher oder ähnlicher Form oder auszugsweise im Rahmen einer anderen Prüfung noch nicht vorgelegt habe.

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