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Review

Role of dietary lignans in the reduction of breast cancer risk

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Lignans are a large group of fiber-associated phenolic compounds widely distributed in edible plants. Some of the ingested plant lignans are converted by intestinal microbiota to enterolignans, enterodiol (END) and enterolactone (ENL), the latter of which has been thought to be the major biologically active lignan, and suggested to be associated with low risk of breast cancer. In line with this, administration of plant lignans which are further metabolized to ENL, or ENL as such, have been shown to inhibit or delay the growth of experimental mammary cancer. The mechanism of anticarcinogenic action of ENL is not yet fully understood, but there is intriguing evidence for ENL as a modulator of estrogen signaling. These findings have generated interest in the use of lignans as components of breast cancer risk reducing functional foods. Identification of target groups, who would benefit most, is of pivotal importance. Therefore, further identification and validation of relevant biomarkers, which can be used as indicators of lignan or ENL action and breast cancer risk reduction at different stages of the disease, are of importance.

Keywords: Breast cancer / Diet / Enterolactone / Estrogen receptor / Lignan

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1 Introduction

Lignans are a heterogeneous group of secondary plant metabolites consisting mainly of phenylpropanoid units. Until now, nearly 500 different lignan structures have been identified from plants. Some oilseeds *e.g.*, flaxseed and sesame seeds have the highest known lignan content of edible plants containing mainly secoisolariciresinol diglucoside (SDG) and sesamin, respectively. In addition to these oilseeds, fiber-rich foods are important dietary source of lignans especially in Western countries. Extensive studies during the past 25 years have revealed the ubiquitous presence of plant lignans, secoisolariciresinol and matairesinol, in different edible plants, *e.g.* seeds, whole grains, vegetables, fruits, and berries [1] as well as in beverages such as coffee, tea, and wine [2, 3]. Based on the content of these two lignans in foods, the estimated intake of dietary lignans in Western countries varies between 0.15 and 1.1 mg *per* day [4, 5]. However, the recent studies have revealed that the edible plants contain also many other plant lignans such as lariciresinol (LAR) and pinoresinol [6], syringaresinol and medioresinol [7, 8], and 7-hydroxymatairesinol [8, 9], and several others in small quantities [8]. This suggests that dietary intake of plant lignans may be higher and the type of compounds more diverse than estimated earlier.

Among European women, breast cancer is the most prevalent cancer accounting for 27% of all new cancers and nearly 18% of cancer deaths of women [10]. Only 5–10% of breast cancers are estimated to be due to genetic predisposition. Several hormonal factors are associated with the higher risk of breast cancer. These include early age at menarche and late menopause, nulliparity, or the first pregnancy after 30 years of age, and long-term hormone-replacement therapy [11, 12]. Also, a number of factors related with life-style and nutrition, and associated with increased breast cancer risk have been identified [13]. These include overweight, high alcohol consumption, and

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Abbreviations: DMBA, 7,12-dimethylbenz[a]anthracene; **END,** enterodiol; **ENL,** enterolactone; **ER,** estrogen receptor; **LAR,** lariciresinol; **MNU,** methylnitrosourea; **SDG,** secoisolariciresinol diglucoside

diet rich in fat and low in fiber. On the other hand, certain life-style and nutritional factors are associated with decreased risk, such as high physical activity and diet rich in fiber, fruits, and vegetables. According to the WHO, dietary factors are estimated to account for approximately 30% of all cancers in Western countries [14]. In European countries with high breast cancer incidence rates, like in Finland, the impact of diet may be even more important as estimated by Finnish Cancer Organisations, stating that up to 60% of the cancers in women are related to diet (http://www.cancer.fi/syovanehkaisy/ravinto/).

Fiber-rich edible plants contain multiple polyphenols, *e.g.* plant lignans. Some of the dietary plant lignans are converted to enterolignans (or mammalian lignans) such as enterolactone (ENL) by gut microbiota. In several epidemiological studies, high consumption of dietary ENL precursors (secoisolariciresinol and matairesinol) or high ENL concentration in serum or urine are associated with lower risk of breast cancer [15–17]. However, there are also conflicting results on the role of lignans, especially ENL, on breast cancer risk [4, 18, 19] which leaves the question of dietary lignans as breast cancer risk reducing compounds still open. Therefore, the experimental studies performed with purified lignans or lignan-rich diets have been of significant importance in elucidating the potential effects and target mechanisms of lignans relevant for breast cancer risk modulation.

2 Experimental mammary cancer models

2.1 The effect of isolated lignans on mammary cancer

Several plant lignans, as well as their mammalian metabolites enterodiol (END) and ENL, have been tested in experimental mammary cancer models *in vivo* (Fig. 1). The most widely studied lignan so far is SDG which has been tested in 7,12-dimethylbenz[a]anthracene (DMBA) and methylnitrosourea (MNU)-induced, estrogen responsive mammary cancer in rats, and in orthotopic, nonestrogen-responsive MDA-MB-435 xenografts in athymic mice (summarized in Table 1). Thompson and coworkers [20] were the first to demonstrate that administration of rats with SDG at a dose (1.5 mg/rat) representing the same amount of lignan as in 5% flaxseed diet, starting 1 wk after the DMBA induction inhibited both the growth in size and the number of tumors. Similar anticarcinogenic effects were seen in late promotion stage starting 13 wk after the DMBA induction and continued for 7 wk [21], suggesting that SDG would at least partially mediate the anticarcinogenic action of flaxseed. SDG has also been demonstrated to reduce the invasiveness of MNU-induced mammary tumors [22] and to inhibit metastasis of human MDA-MB-435 breast cancer cell-derived tumors [23]. So far, several other plant lignans, *e.g.*, arctiin, 7-hydroxymatairesinol, LAR, and sesamin

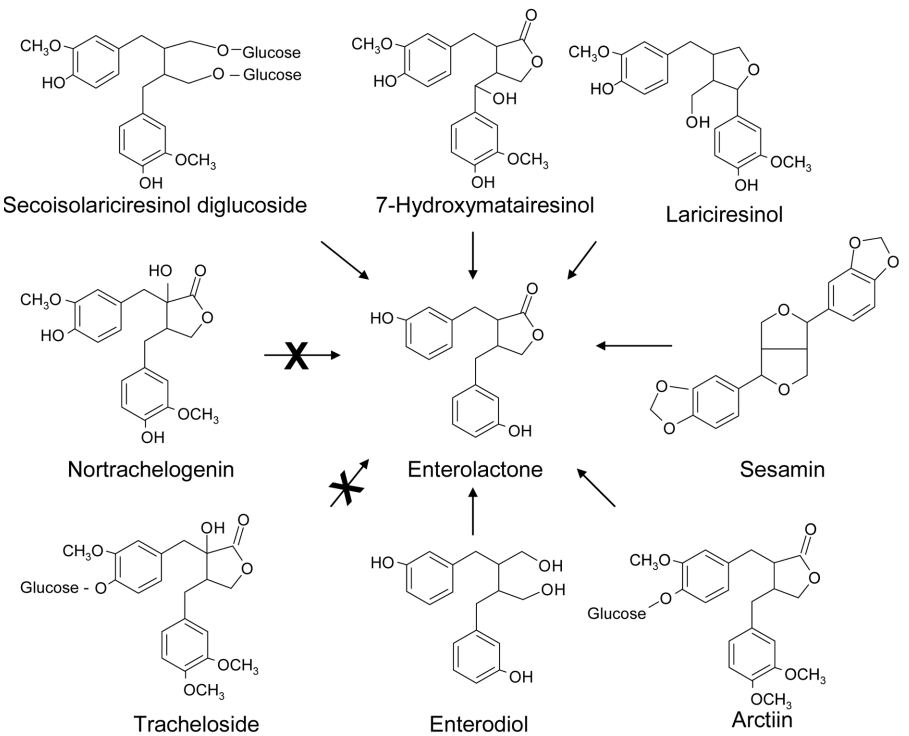

Figure 1. Lignans tested in *in vivo* cancer models, and their convertibility to ENL by intestinal microflora (indicated by arrows).

Table 1. Effects of purified lignans on mammary cancer *in vivo*

Administered lignan	Mammary cancer model	Treatment time and dose	Effect	Reference
Arctiin	PhIP-induced SD rats	0.2 or 0.02% in diet during or after initiation	Reduced tumor multiplicity after initiation, no difference in incidence	[24]
ENL	DMBA-induced SD rats	Weeks 9 – 16 after initiation, 1 or 10 mg/kg b.w./day	Reduced growth of new and established tumors	[30]
END and ENL	Ovariectomized athymic Balb/c nu/nu mice bearing orthotopic MCF-7 tumors	22 wk after removal of E2 pellet, 10 mg/kg b.w./day	Reduced tumor size similar to negative control	[59]
7-Hydroxymatairesinol	DMBA-induced SD rats	Weeks 9 – 16.5 after initiation, 15 mg/kg b.w./day	Reduced tumor volume and growth, no effect on multiplicity	[26]
7-Hydroxymatairesinol	DMBA-induced SD rats	Started 1 wk before initiation, or weeks 9 – 17 after initiation, 4.7 mg/kg b.w./day	Reduced tumor volume and growth, no effect on multiplicity	[27]
Lariciresinol	Ovariectomized, E2 pelleted athymic Balb/c nu/nu mice bearing orthotopic MCF-7 tumors	5 wk after tumors were established, 20 or 200 mg/kg diet	Reduced growth of the tumors	[28]
Nortrachelogenin	DMBA-induced SD rats	Weeks 10 – 18 after initiation, 15 mg/kg b.w./day	No difference in tumor volume or multiplicity	[32]
Secoisolariciresinol diglucoside	DMBA-induced SD rats	Weeks 1 – 20 after initiation, 1.5 mg/day	Reduced number and multiplicity of mammary tumors	[20]
Secoisolariciresinol diglucoside	DMBA-induced SD rats	Weeks 13 – 20 after initiation, 1.5 mg/day	Reduced tumor volume, number and incidence of new tumors	[21]
Secoisolariciresinol diglucoside	MNU-induced SD rats	Weeks 1 – 22 after initiation, 0.7 or 1.4 mg/day	Reduced tumor invasiveness. With the higher dose reduced tumor multiplicity, with lower dose incresed tumor multiplicity	[22]
Secoisolariciresinol diglucoside	Athymic Ncr nu/nu mice bearing orthotopic MDA-MB-435 tumors	7 wk after removal of primary tumor, 0.2 g/kg diet	Reduced incidence of metastasis, no significant difference in tumor recurrence	[23]
Sesamin	DMBA-induced SD rats	Starting 1 wk before initiation for 12 wk, 0.2% in diet	Reduced number of tumors	[25]
Tracheloside	PhIP-induced SD rats	0.2 or 0.02% in diet during or after initiation	No differences in incidence, multiplicity, or volumes	[31]

DMBA, 7,12-dimethylbenz[a]anthracene; E2, 17-beta-estradiol; MNU, methylnitrosourea; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; SD, Sprague – Dawley.

have also been demonstrated to inhibit mammary tumorigenesis (Fig. 1, Table 1). Administration of arctiin or sesamin to rats during and post carcinogen induction reduced the mammary tumor multiplicity and numbers [24, 25] while administration of 7-hydroxymatairesinol to rats during or post DMBA-induction reduced the tumor growth in size but did not significantly alter the multiplicity of the tumors [26, 27]. Accordingly, in MCF-7 tumor bearing mice, dietary LAR (20 or 100 ppm) decreased the estradiol triggered tumor growth (volume and weight) by increasing the cell apoptosis [28]. These findings suggest that different plant lignans have distinct mechanisms of antitumorigenicity in carcinogen-induced rat mammary tumor models. Interestingly, all these lignans can be converted to ENL or both END and ENL [7, 9, 26, 29]. ENL, given as a pure compound, has been demonstrated to inhibit the growth in volume of estrogen responsive mammary tumors in intact rats [30], suggesting that it may, at least partially, account for the antitumorigenic properties of these plant lignans. This hypothesis on the importance of ENL formation for

inhibition of mammary tumor development is further supported by the lack of anticarcinogenic effects of structurally closely related plant lignans, *e. g.*, nortrachelogenin and tracheloside, which both have a hydroxyl group at C-8 making them nonconvertible to ENL (Fig. 1). Neither of the compounds inhibited the growth in volume or multiplicity of the carcinogen-induced mammary tumors in rats [31, 32]. However, it is important to realize that in addition to enterolignans, plant lignans may also be absorbed in considerable quantities [33] and they may undergo enzymatic transformations in the liver [34, 35]. In addition, ingested lignans may also undergo other chemical transformations which do not involve intestinal bacteria or hepatic enzymes [36]. Thus, plant lignans *per se*, or their further metabolites may as well contribute to the tumor growth and have compound-specific mechanisms on the tumorigenesis.

2.2 The relevance of experimental models for human breast cancer and usefulness for cancer chemoprevention studies

A critical question is the relevance to human disease of the results obtained in experimental cancer models. The *in vivo* models currently in use all have their advantages and limitations. Hormone dependence is the most important advantage of the DMBA and MNU-induced mammary cancers. Like the majority of human breast cancers, most of the DMBA and MNU-induced mammary tumors are adenocarcinomas [37, 38], express estrogen and progesterone receptors, and are stimulated by physiological doses of estrogens [39, 40]. There are also clear differences. One of the striking dissimilarity in the DMBA-induced rodent mammary carcinoma is its prolactin dependence. Prolactin is essential for DMBA-induced tumors growth in rats while it is not known to be as important in humans. Prolactin inhibitors regress tumors very effectively in rats, but in clinical trials systemic prolactin inhibitors were not associated with any clear remission among patients treated only with these compounds [41]. Another important difference compared to human breast cancer is the rare metastasis of the DMBA and MNU-induced tumors. Therefore, these models are not suitable for studying the compounds which primarily influence invasion or metastasis.

In the rodent models, plant-based natural ingredient diet given during the initiation with carcinogen inhibits or delays tumor development, compared to purified (semisynthetic) diet [26, 27, 42, 43]. This is likely to be due to reduction in procarcinogen activation (as DMBA needs first to be metabolized in the body to an active carcinogen), without any obvious involvement of estrogen receptor (ER)-mediated effects. Even though it is tempting to speculate about a role for polycyclic aromatic hydrocarbons in carcinogenesis, they have never been confirmed to be involved in human breast cancer etiology [44], and the human relevance of this finding remains unclear.

Xenograft models using human cancer cells transplanted to immunodeficient mice offer certain advantages over the carcinogen-induced models. Firstly, tumors are of human origin, and maintain the key aspects of hormone dependence and growth regulation of human breast tumors, including in some cases also the metastatic activity. Secondly, this approach allows genetic modification of the cells prior to transplantation, giving powerful tools for the investigation of the specific roles of selected receptors, other transcription factors, growth factors, and steroid metabolizing enzymes. However, the models of human breast cancer cell tumors in mice to mimic human disease are not without limitations. The breast cancer cell lines used for xenografts are adapted to *in vitro* culture conditions which may have affected their molecular and cellular characteristics and equivalency to primary human disease. In addition, the necessity to use immunodeficient animals, which lack the production of T-cells, as hosts for human cancer cell transplants does not take into account the possible T-cell-derived immunomodulatory responses against the disease progression [45]. Moreover, xenograft models are not suitable to test the diet effects on primary and secondary prevention of breast cancer (*i. e.*, phases prior to cancer initiation or its diagnosis). Instead, these models, similar to carcinogen-induced rodent models, can primarily be applied to test the diet effects on cancer promotion and progression (*i. e.*, tertiary prevention).

As regards studies with dietary plant phenolics in the rodent models, one of the key questions is the similarity of metabolism of the ingested plant phenolics in the gut. In rats, oral administration of plant lignans LAR, matairesinol, secoisolariciresinol, 7-hydroxymatairesinol [33], and sesamin [29] have been shown to increase the urinary excretion of ENL. *In vitro* fermentation studies with human fecal microbiota have also demonstrated ENL production from these plant lignan precursors [7, 29]. Accordingly in human subjects, ingestion of isolated SDG, sesamin, or 7-hydroxymatairesinol have been reported to increase the urinary and/or serum enterolignan concentrations (Hormos Medical Corp., 2004, New dietary ingredient notification for 7-hydroxymatairesinol (HMR) potassium acetate complex, http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0235-05-Contents-vol167.pdf) [9, 46].

Immune-deficiency of athymic mice has been suggested to affect their gastrointestinal microbiota and the ability to form enterolignans from plant lignans precursors. Apparently, this is not the case, as male athymic mice on LAR-containing feed have high concentration of END and ENL in the serum (Fig. 2) and the concentration of enterolignans is higher in animals receiving 100 ppm LAR diet, compared to 20 ppm LAR diet. These studies indicate that rats, as well as athymic mice, like humans, are able to convert purified plant lignans precursors to enterolignans despite the obvious difference in their gut microbiota.

In addition to plant lignans, degradation of plant fiber lignins has also been suggested to contain precursors for

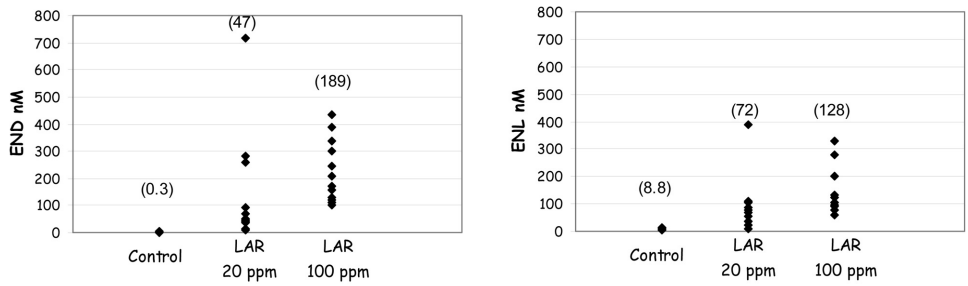

Figure 2. Enterolignan concentrations in serum of adult male athymic Balb/cABom mice. The mice were fed with semisynthetic AIN-93G diet (=control), or AIN-93G diet containing lariciresinol 20 or 100 ppm (LAR 20 and LAR 100, respectively) for 8 wk. ENL and END concentrations were determined by LC-MS/MS method as described previously [36]. Each symbol represents one animal. The median of the results is expressed in parenthesis on the top of each group. Number of animals: 11 (control), 12 (LAR 20 ppm), 12 (LAR 100 ppm).

mammalian lignan production. Based on the current knowledge, rodents are able to produce enterolignans from the cereal bran plant lignin polymers [47]. However, in human subjects the consumption of lignin-rich fiber did not increase the urinary enterolignan excretion [48] indicating that there may be fundamental differences in enterolignan production from lignin polymers between rodents and man.

3 Lignans in breast cancer prevention – Putative mechanisms and targets of action

Epidemiological studies and experimental studies (*in vivo* mammary cancer models) support the idea of lignans as chemopreventive agents. The mechanisms of lignan action relevant for breast cancer chemoprevention are, however, still very unclear. In several occasions, it has been suggested that lignans would exert their action *via* ERs, primarily acting as antiestrogens, even though there is very little, if any, concrete evidence to support this hypothesis.

Results from *in vivo* studies with various experimental mammary cancer models are well in line with each other, showing that oral exposure to lignans (arctiin, ENL, 7-hydroxymatairesinol, LAR, SDG, and sesamin), either as purified compounds (reviewed above), or as components of complex plant matrix (flaxseed), delayed or inhibited growth of the tumors in size or reduced the multiplicity. On the other hand, these and other *in vivo* studies [26, 49] show that lignans do not induce classical estrogenic or antiestrogenic effects in female or male rodent reproductive organs (*e.g.*, uterine growth in immature females, or regression of male accessory sex glands). However, dietary flaxseed or SDG supplementation of adult virgin or pregnant rats induced changes in estrous cycle and resulted in persistent estrogen-like effects in the offspring [50, 51]. Part of the endocrine modulatory effects of flaxseed diet may thus be due to flaxseed lignan SDG or its further metabolites such as ENL or other flaxseed components, such as the oil or fiber.

Most mechanistic studies have focused on ENL as the biologically active lignan. There are several *in vitro* studies showing that high concentration (≥1 μM) of ENL may exert estrogen-like actions in human cancer cells, including the induction of endogenous estrogen-regulated genes [52, 53], as well as estrogen responsive element (ERE, promoter) containing reporter gene ([54]; Penttinen *et al.*, unpublished results). However, the relative binding affinities of ENL to ERα and ERβ are low (0.07 and 0.01 compared to diethylstilbestrol, respectively) [54], and, thus, the biological relevance of these *in vitro* findings remains to be confirmed.

However, it should be noted that in certain individuals, in particular those consuming flaxseed or sesame seed, concentration of enterolignans (ENL and END) in serum may be well over 1 μM [8, Saarinen *et al.*, unpublished results], and concentration of ENL in breast tissue may exceed that in the serum [55]. It is thus pertinent to ask if ENL, indeed, may act as a modulator of estrogen action *in vivo*, given the current knowledge of its "physiological concentrations" and the diversity of ER functions, depending on the target cell and tissue properties, such as the endogenous hormonal milieu, and expression of ER subtypes and the ligand-selective ER coregulators.

3.1 ENL regulates the proliferation of estrogen-sensitive breast cancer cells in an ER-dependent manner

We recently confirmed that ENL regulates the proliferation of estrogen-sensitive MCF-7 cells *in vitro*. ENL, at concentrations ≥ 1 μM, significantly stimulated cell proliferation, and the effect was abolished by known antiestrogens, tamoxifen and ICI-182,780 (Fig. 3). When ENL was added in combination with 17β-estradiol (E2), no reduction in cell number was seen, indicating that ENL did not act similar to antiestrogens. The latter finding is somewhat contradictory to the results published earlier by Mousavi and coworkers [56], showing that cotreatment with ENL and E2 resulted in

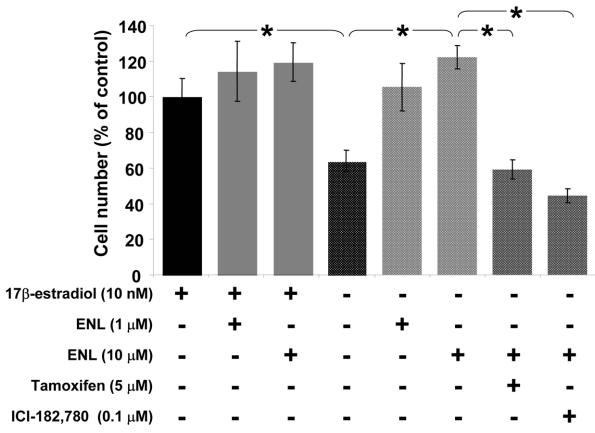

Figure 3. Effect of ENL on the proliferation of MCF-7 breast cancer cells. Cells were cultured for 7 days in the presence of the test compounds or vehicle. Number of viable cells was quantified with WST assay [77]. Statistically significant differences between the treatment groups are denoted with an asterisk (*).

attenuation of the proliferative response in MCF-7 cells. At present, there is no explanation for these conflicting results.

The findings that ENL stimulates MCF-7 cell proliferation, and that the effect can be abolished by a pure ER antagonist ICI-182,780, suggest that ENL acts as an ERα agonist, as MCF-7 cells express predominantly ERα, which mediates the proliferative effect of estrogen agonists in these cells. These results are also in accordance with the earlier studies demonstrating that ENL upregulates the expression of endogenous ER-regulated genes, pS2 and progesterone receptor, in MCF-7 cells [52, 53].

There are a number of dietary plant-derived phenolic compounds and their mammalian metabolites that have been shown to stimulate the proliferation of estrogen-responsive breast cancer cells. Would these results then indicate that growth stimulation of breast cancer would occur also *in vivo*, or should these results be taken merely as an indication of the ability of certain phenolics to transactivate ERα, and to induce estrogen-like effects similar to endogenous estrogen? So far, only a very small number of phytoestrogens have been tested in xenograft models, to assess the putative effects on breast cancer cell proliferation *in vivo*. The results clearly show that prediction of *in vivo* responses based on *in vitro* results is not necessarily reliable. For example, genistein stimulates proliferation of MCF-7 cells both *in vitro* and *in vivo* [57], while with equol (metabolite of daidzein) and ENL no induction of MCF-7 cell xenograft growth was observed [58, 59], despite their stimulatory effect on the same cells in culture (Fig. 3). Again, this points out the necessity of *in vivo* testing, if the endpoint of interest is regulation of tumor growth.

3.2 Interaction of ENL with ERs

The ability of ENL to transactivate ERs and its possible ER-subtype selectivity are intriguing questions, as several other phenolic phytoestrogens (*e.g.*, genistein) have been demonstrated to transactivate ERs relatively efficiently, and to have preference for ERβ over ERα [60]. So far, there are two published studies addressing this question: Mueller and coworkers [54] demonstrated that in Ishikawa cells transfected either with ERα or ERβ, and an ERE-driven reporter gene, ENL induced the reporter gene expression only marginally (relative potency 0.01 and 0.007, and relative efficacy 49 and 77, for ERα and ERβ, respectively). Furthermore, we have previously shown that in HEK293 cells ENL had no significant effect on either ERα or ERβ transactivation below 10 μM [26]. This is quite puzzling, in the light of the *in vitro* studies (described above) showing that ENL stimulates the expression of endogenous estrogen- and ER-mediated responses at 1 μM concentration. Obviously, it should be taken into account that ER transactivation by a given ligand/compound depends on several factors in addition to the binding affinity to the receptor. Thus, the degree of a reporter gene induction by the very same ligand may vary between different cells, depending on, *e.g.*, the properties of the reporter gene construct, the availability and recruitment of different ER-coregulators, and the metabolic activity of the cell. In line with this, we have recently demonstrated that ENL efficiently induces ERα-mediated transcription in certain cell lines, while it is far less potent in others, suggesting that ENL acts as an ER agonist in cell-specific manner (Penttinen *et al.*, manuscript submitted).

Clearly, there is still a discrepancy between *in vitro* and *in vivo* studies, the latter showing no indication for ERα-mediated effects, despite the presence of high concentrations of ENL. So far, most *in vivo* studies have focused on traditional/classical estrogen effects (*e.g.*, uterine growth and inhibition of central nervous system-gonadal axis), which may explain the lack of observed effects. Indeed, we have recently obtained evidence that ENL acts as an ER agonist in gene- and tissue- selective manner in ovariectomized mice (Penttinen *et al.*, manuscript submitted). Further studies are thus warranted to clarify the whole spectrum of ER-mediated actions of ENL *in vivo*. Would these effects explain the tumor growth inhibiting properties of ENL, remains to be shown.

3.3 Non-ER-mediated actions relevant for breast cancer prevention

In addition to interaction with ERs, lignans may modulate estrogen action *via* other mechanisms, such as inhibition of estrogen biosynthesis. ENL, and some plant lignans have been shown to inhibit aromatase *in vitro* [61–63], but, again, the relevance of these findings *in vivo* has not been confirmed. ENL, even though it is the most potent aroma-

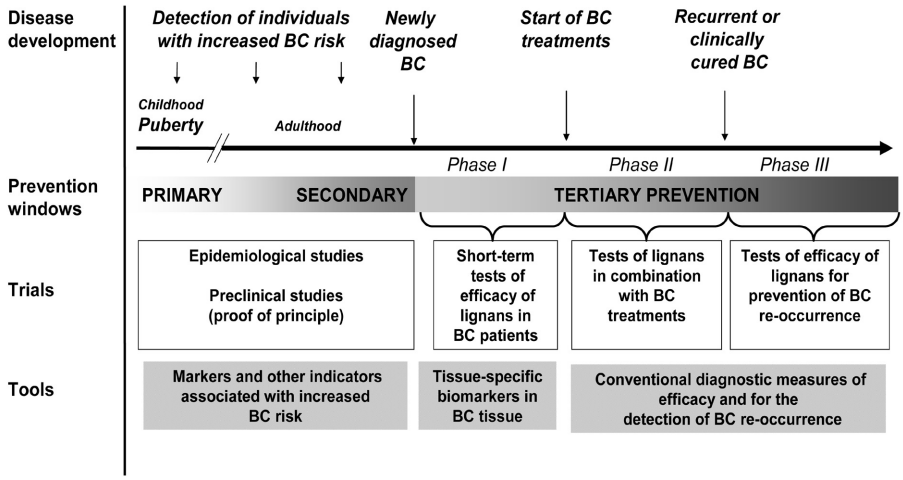

Figure 4. Different phases in breast cancer (BC) risk reduction – selected windows of opportunity for dietary lignans.

tase inhibitor of lignans tested so far, its inhibitory activity is only modest, at best (EC_{50} 8.9 μM) [62]. It is thus quite unlikely that ENL, or other lignans, would inhibit ovarian estrogen production, as the aromatase activity in gonads is very high. Therefore, the most interesting question is whether ENL would be able to modulate the target cell intracrinology, *i.e.*, inhibit local aromatization in normal breast tissue or breast cancer, or whether ENL could reduce estrogen production in other extragonadal sites (*e.g.*, adipose tissue) in postmenopausal women. Unfortunately, studies to demonstrate a diet-related change in extragonadal aromatization in human tissues are very difficult to conduct. Moreover, the rodent models are not very well suited for this purpose, because extragonadal aromatization plays only a minor role in their estrogen biosynthesis.

In addition to direct modulation of estrogen levels and genomic ER-mediated actions in mammary gland and breast cancer cells dietary compounds may affect other signaling pathways independent or downstream of ERs, *e.g.*, by inhibiting angiogenesis [64], tyrorosine kinases *via* IGF-I receptor and C-erbB2/HER2/neu mediated pathways [65], other epidermal growth factor-family members [55, 66], COX-2 expression [67], or DNA topoisomerase II [68]. Moreover, new target genes are likely to be identified using gene expression microarrays.

4 Breast cancer prevention – Windows of opportunity for lignans and lignan-containing functional foods

Breast cancer prevention can be divided into three phases: primary, secondary, and tertiary prevention (Fig. 4). Primary prevention can occur during childhood, adolescence, and adulthood. Secondary prevention can be defined as a time window when markers of premalignant changes in mammary gland are already present, such as increased breast density [69, 70], which reflect the increased breast cancer risk but cancer is not yet diagnosed. In this setting, epidemiological as well as preclinical studies offer important information on the factors associated with both increased and decreased risk. Biomarkers or surrogate markers for premalignant changes and breast cancer would be necessary for the demonstration of the role of selected dietary or serum lignans in reduction of breast cancer risk, and, in the case such evidence would be obtained, would justify the formulation of generic health claims for disease risk reduction. However, currently, the lack of specific and validated biomarkers, which are causally related to the development of the disease, is one of the major challenges for assessment of commonly accepted principles for primary breast cancer prevention. The tertiary prevention window of breast cancer focuses on prevention of disease progression and/or recurrence. This window contains at least three separate phases on which the effects of lignan-rich diets or functional foods can be investigated. In the first tertiary prevention phase, short-term tests for efficacy can be performed in patients with newly diagnosed breast cancer who are waiting for the start of the breast cancer treatments. The main advantage of this phase is the availability of tumor tissue for analysis of tissue-specific biomarkers both before and after the dietary intervention. The second phase of tertiary prevention occurs at the stage of disease when the breast cancer treatments have already been started. During this phase, the combination effects of dietary lignans with breast cancer therapy can be investigated, *i.e.*, the role of dietary factors in combination with medical treatments can be elucidated. The preclinical findings on the interaction effects of lignans with the current breast cancer drug therapies indicate that dietary lignans may attenuate the development of drug resistance against drug treatments and thus contribute to inhibition of cancer progression [71]. The last phase of tertiary prevention occurs after the primary disease has been clinically cured and focuses on the prevention of breast cancer recurrence. For assessment of the treatment efficacy in the two latter phases, conventional measures for the detection of breast cancer recurrence can be applied. The

possibility to investigate the effects of dietary lignans or lignan-containing functional foods on tertiary prevention of breast cancer would generate product-specific information on efficacy and therefore would justify the use of a product-specific health claim.

Because of the findings from preclinical studies on the effects of different lignan compounds on mammary cancer, the research on dietary lignans is currently focused on the compounds that can be further converted to enterolignans, *e.g.*, ENL. The epidemiological data on the associations between serum and urine lignan concentrations and breast cancer risk are accumulating, and it can be used to support the hypothesized role of lignans as breast cancer risk reducing agents. However, the epidemiological data are not concurrent [72] and did not prove causal relationship between ENL and breast cancer risk, and, thus, leaves the role of lignans in primary and secondary breast cancer prevention largely open. Nevertheless, animal studies investigating the effects of early life exposures to plant lignans or lignan-rich diet on later development of breast cancer [66, 73–75] suggest that consumption of lignans (*i.e.*, SDG) prepubertally may enhance the mammary gland maturation and thus affect the breast cancer susceptibility at later age. Whether this modulation of mammary gland development is due to the plant lignans, or their mammalian metabolites still remains to be explored.

The effects of lignans or lignan-rich diets in tertiary breast cancer prevention (Table 1) as such, or as adjuvant for drug therapy [71] in experimental cancer models, have provided promising results. Until now, there is only one human study published on the effects of lignan-rich food, *e.g.*, flaxseed muffin, in newly diagnosed breast cancer patients waiting for surgical operation [76]. In line with the experimental findings, this pilot-scale study suggests that the dietary intervention was associated with favorable changes in tumor properties (*e.g.*, reduced expression of proliferation markers). Obviously, clinical trials using methodologically and biologically validated biomarkers are necessary for the development of breast cancer risk reducing functional foods. Lignans are not the magic bullet to prevent breast cancer, but as a part of a healthy diet and lifestyle they might help to reduce breast cancer risk in general population. Understanding of the mechanisms of action of lignans is necessary to find the right setting for the prevention studies. Furthermore, in the future, when more information is available from the clinical trials, specific target groups who would benefit most from these products (*e.g.*, individuals with specific markers for increased breast cancer risk) may be recognized. This would allow development of the lignan-containing functional foods for those selected high-risk groups at a specific prevention window.

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