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Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts

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Abstract

Angiogenesis is important in tumor growth, progression and metastatic dissemination. Vascular endothelial growth factor (VEGF) is one key factor in promotion of breast cancer angiogenesis. VEGFs are bioactive in the extracellular space where they become available to the endothelial cells. Phytoestrogens such as lignans have been shown to alter breast cancer incidence and be cancer-protective in rats. We show that supplementation of 10% flaxseed, the richest source of mammalian lignans, to nude mice with established human breast tumors reduced tumor growth and metastasis. Moreover, flaxseed decreased extracellular levels of VEGF, which may be one mechanistic explanation to the decreased tumor growth and metastasis.

Keywords: MDA-MB-435; Microdialysis; Flaxseed; Metastasis; Vascular endothelial growth factor; Angiogenesis

1. Introduction

The incidence of breast cancer differs in various populations of the world and migrant studies indicate that the difference is largely attributable to environmental factors rather than genetics [1,2]. The plant-based diets with high contents of phytoestrogens such as lignans and isoflavonoids have been proposed to be responsible for the low breast cancer incidence in Asian women but the mechanisms are unexplored. Experimental animal studies have supported the epidemiological data and shown a chemopreventive effect of phytoestrogens on mammary, colon, and skin tumors [3,4]. Administration of phytoestrogens to newborn rats delays the onset of mammary cancer after carcinogen treatment and exposure to lignans induces structural changes in the highly proliferative terminal buds leading to atrophy of the mammary gland [5–7]. Flaxseed (FS) is the richest source of secoisolariciresinol diglycoside (SDG), the main precursor of mammalian lignans. Colonic bacteria converts SDG to the two major mammalian lignans, enterodiol (ED) and enterolactone (EL) [8,9].

Angiogenesis, the generation of new capillaries from preexisting vessels, is one of the most important events in tumor growth, progression and metastatic dissemination [10,11]. Vascular endothelial growth factor (VEGF) appears to be a key factor in promotion of tumor angiogenesis and in breast cancer VEGF mRNA expression is increased compared to adjacent normal breast tissue [12]. Moreover, high VEGF levels correlate with poor prognosis and decreased overall survival for both node-positive and node-negative breast cancer patients [13,14]. VEGFs are bioactive as freely diffusible proteins in the extracel-

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lular space where they become available to the endothelial cells, and it has been suggested that the soluble isoform has greater angiogenic and tumorigenic properties than the heparin bound isoforms [15].

The estrogen receptor-negative human breast carcinoma cell line, MDA-MB-435, has been shown to develop metastasis in lymph nodes, lungs, and other visceral organs after tumor formation in the mammary fat pad in nude mice [16–18]. In contrast to other cell lines, which exhibit a low metastatic ability, MDA-MB-435 tumors produce distant metastases in 80–100% of recipients and is therefore a tool for analysis of the cellular and molecular basis of the metastasis of advanced breast cancer [19].

The objective of this study was to determine whether a diet supplemented with FS to nude mice with established MDA-MB-435 tumor explants affected tumor growth, metastasis, and extracellular VEGF.

2. Materials and methods

2.1. Cell line and animals

The estrogen receptor-negative MDA-MB-435 human breast cancer cell line was obtained from Dr Janet Price, Department of Cell Biology, M.D. Anderson Center, Houston, TX. The cells were maintained in Iscove's modified Dulbecco's medium supplemented with 5% fetal bovine serum, plus penicillin and streptomycin (Sigma, St. Louis, MO).

Twenty female athymic nude mice (NCR-nu/nu), 3–4 weeks of age, were purchased from Simonsen Laboratories (Gilroy, CA) and maintained in a microisolator within a pathogen free isolation facility. They were fed with sterilized diet and autoclaved water ad libitum. Surgical procedures were performed under aseptic conditions in a laminar flow hood. The University of Toronto Animal Care Committee approved all animal work.

2.2. Experimental design

The mice were acclimatized for 1 week, while being fed the basal diet (BD). They were anesthetized with ketamine/xylazine and MDA-MB-435 cells (1×10^6 in 50 μl of culture medium) were injected into the surgically exposed right-sided thoracic mammary fat pad. The inoculation sites were palpated at weekly intervals. The maximum diameters and perpendicular diameter of the palpable mammary fat pad tumors were measured and the surface areas were calculated by the following equation ($L/2 \times W/2 \times 3.14$). At week 8, mice were randomized into two groups, such that the mean body weight and tumor size in each group were the same, and fed for 6 weeks with either BD or 10% FS diet. Palpable tumor size was monitored weekly as before.

At necropsy, the body and mammary fat pad tumor weights were recorded. The extent of macroscopic metastasis was assessed using a dissecting microscope at 40× magnification.

2.3. Diets

Two different diets were prepared: BD, which is based on AIN93G [20] modified to contain 20% corn oil, or BD supplemented with 10% ground FS (Linott variety; Omega Products, Melfort, Saskatchewan, Canada). The FS diet was corrected for the FS's contribution to fat, carbohydrate and protein components so that the two diets were isocaloric. Both diets were prepared by DYETS Inc. (Bethlehem, PA) and sterilized by gamma irradiation by Isomedix Corp. (Whitby, Ontario, Canada).

2.4. Microdialysis equipment and experiment

Microdialysis is a minimal invasive technique that allows continuous sampling of the extracellular fluid in a specific organ or tissue [21,22]. Diffusion of low molecular substances over the dialysis membrane is almost complete at low flow rates [21]. However, for larger molecules such as VEGF the recovery over the membrane decreases. To estimate the recovery of VEGF, probes were calibrated in vitro by dialysis of VEGF standard solutions; the mean recovery at room temperature was $4 \pm 0.06\%$ at a flow rate of 2 μl/min. After a 30 min equilibration period the levels of VEGF did not change over time for a period of 5 h when the flow rate was constant. However, the in vitro recovery can only be an estimate of the in vivo recovery since other factors such as tissue pressure and temperature will affect the diffusion of substances. Therefore, all microdialysis values are given as original data without re-calculations.

Fourteen weeks after the tumor cell injections tumor-

bearing mice, ten in each group, were anesthetized with an i.p. injection of ketamine/xylazine and thereafter kept anesthetized by repeated s.c. injection with 25% of the initial dose of ketamine/xylazine. A heating pad maintained body temperature. A small skin incision was made and microdialysis probes (CMA/20, 0.5 mm diameter; PES membrane length 4 mm, 100 kDa cut-off, CMA/Microdialysis, Stockholm, Sweden) were inserted into tumor tissue and sutured to the skin. The complete length of the 4 mm membrane was situated in tumor tissue, i.e. all tumors were larger than 4 mm. The probes were connected to a CMA/102 microdialysis pump (CMA/Microdialysis, Stockholm, Sweden) and perfused at 2 μl/min with a physiological solution containing 147 mmol/l Na^+, 4 mmol/l K^+, 2.3 mmol/l Ca^{2+}, and 156 mmol/l Cl^-. After a 30 min equilibration period, the outgoing perfusate was collected for 4 h on ice and stored at −70 °C for subsequent analysis.

2.5. VEGF analysis

All samples were analyzed using a commercial quantitative immunoassay kit for human VEGF (Quantikine, human VEGF; R&D systems, Minneapolis, MN). According to the manufacturer this kit measures the soluble VEGF 165 and 121 isoforms and the sensitivity is less than 5 pg/ml; intra-assay and inter-assay precision is 4.5–8.8%. All samples were assayed in duplicate in the same assay and the intra-assay precision given by the manufacturer was confirmed in our lab.

2.6. Statistics

The data are expressed as means ± SEM. Student's *t*-test, Fisher's exact test, and simple regression were used as appropriate. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Body weight and food consumption

No significant difference in initial body weight, food intake or body weight change were recorded between the treatment groups. Weights of major organs, such as the liver, heart, spleen, kidney, ovary and uterus, adjusted to body weight, were not significantly different (data not shown).

3.2. Tumor growth

Tumor growth rate, the effect of treatment over time, was significantly lower in tumors exposed to 10% FS, i.e. 10.2 ± 2.4 vs. 16.6 ± 1.7 mm^2/week in the BD group ($P < 0.05$, $n = 10$ in each group) (Fig. 1). None of the tumors in the flax group went into complete regression but all had a slower growth rate than the tumors in the BD group.

3.3. Metastasis

The incidence of macroscopic distant metastasis, lungs, distant lymph nodes, and other organs was significantly decreased in mice fed with 10% FS, i.e. 1/10 compared to 7/10 in the BD group ($P < 0.05$) (Table 1). The number indicates animals with one to several metastases.

3.4. Effects of 10% FS on extracellular release of VEGF

The levels of VEGF in extracellular fluid were decreased in FS-exposed tumors compared with BD tumors, i.e. 10 ± 3 vs. 22 ± 8 pg/ml. However, the difference was statistically significant only in the large tumors >2.5 g, i.e. 5 ± 5 pg/ml in the FS

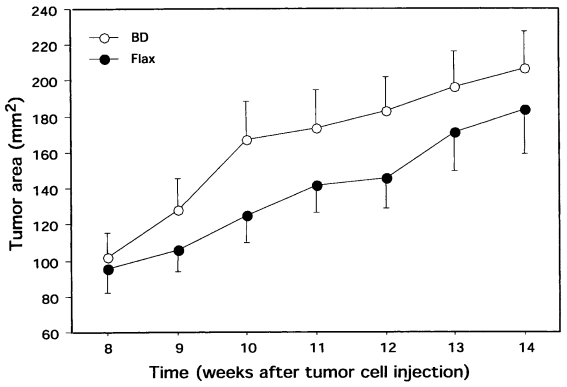

Fig. 1. Effects of a diet supplementation of 10% FS on tumor growth of MDA-MB-435 human breast cancer xenografts in nude mice. Eight weeks after tumor cell injection into mammary fat pads the mice were randomized to two groups receiving either BD or a supplement with 10% FS. The tumor growth rate was decreased in mice fed with 10% FS ($P < 0.05$, $n = 10$ in each group).

Table 1
Effect of 10% FS diet on distant metastasis in nude mice with MDA-MB-435 human breast tumor xenografts[a]

Diet	Metastasis (%)	No metastasis
BD	7/10 (70)	3/10 (30)
10% FS	1/10 (10)	9/10 (90)

[a] Athymic nude mice were injected with MDA-MB-435 human breast adenocarcinoma cells into the mammary fat pad. Eight weeks after tumor cell injection the mice were randomized into two groups with similar tumor size and body weight. One group was fed with BD while the other was supplemented with 10% FS. Six weeks after diet supplementation the mice were killed and the number of metastases was assessed by a dissecting microscope and later confirmed by histology. There was a statistically significant difference in the incidence of metastasis between the groups. Fisher's exact test was used for statistical analysis ($P < 0.05$).

group compared to 60 ± 10 pg/ml in the BD group ($P < 0.05$, $n = 3$ in each group) (Fig. 2).

3.5. Association between extracellular VEGF and tumor size

Comparison of the extracellular VEGF with tumor size revealed a significant correlation between the parameters in the BD diet group ($P < 0.05$), but not in the FS group (Fig. 3A,B).

4. Discussion

In this study we show that a dietary supplementation with 10% FS decreased the tumor growth rate and metastasis of estrogen receptor-negative breast tumor explants in athymic nude mice. Moreover, we show, for the first time, that the extracellular levels of VEGF in large tumors were significantly lower after a FS diet compared to BD.

VEGF acts on endothelial cells by stimulating cell proliferation, migration, and tubular organization and increases vascular permeability [23]. To exert its effect VEGF has to be secreted into the extracellular fluid. Several isoforms of human VEGF containing 121, 165, 189, and 206 amino acids are produced from a single gene as a result of alternative splicing [24]. The various isoforms differ in the affinity for heparin and heparin-like molecules, which affect their bioavailability [25]. Previous assessments of VEGF protein have been performed by immunohistochemistry or immunoassay of tissue extracts and these measurements appear to correlate with microvessel density at least in invasive ductal carcinoma of the breast [26,27]. However, VEGF 121, considered to be the most potent stimulator of angiogenesis in vivo and the predominate isoform in primary human breast cancer, diffuses freely into the extracellular space from the cells producing it and cannot be detected by immunostaining of tumor sections [15,28]. The longer isoforms of VEGF may be released in soluble bioactive form by proteolytic cleavage [23]. This cleavage may be especially important in tumors where the local microenvironment generally express a high proteolytic activity [29]. Clearly, a direct measurement of VEGF locally in the tumor would be more accurate for measurement of bioactive

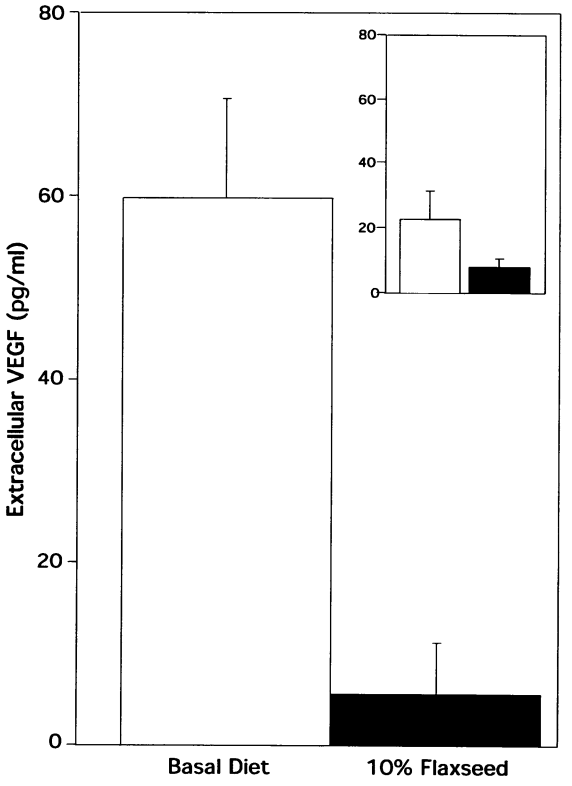

Fig. 2. Effects of a diet supplementation of 10% FS on extracellular levels of VEGF in MDA-MB-435 human breast cancer xenografts in nude mice, measured with microdialysis. Large tumors exposed to 10% FS had decreased levels of VEGF compared to tumors exposed to BD ($P < 0.05$, $n = 3$ in each group). The inset shows the difference in tumors of all sizes.

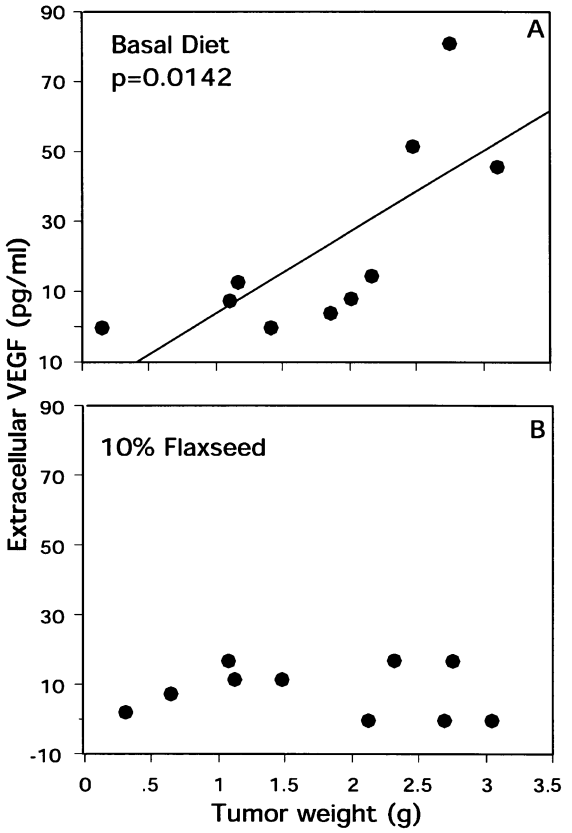

Fig. 3. Effect of 10% FS diet on the correlation between extracellular VEGF levels, measured with microdialysis, and tumor size in nude mice with MDA-MB-435 human breast tumor xenografts. (A) The parameters correlated significantly in mice fed with BD. (B) In mice fed with 10% FS the expected increase of extracellular VEGF in large tumors failed to occur.

protein released by the tumor. Our results indicate that microdialysis is a useful technique for detection of VEGF in tumor tissue in vivo.

Hypoxia seems to be an important stimulus for inducing VEGF mRNA expression in human mammary tumors mainly through hypoxia-inducible factor-1 (HIF-1) [30,31]. Moreover, increased levels of HIF-1 alpha have been associated with more aggressive breast tumors [32]. Our results show that in the BD group VEGF correlated significantly with tumor size which suggests that the larger the tumor the more hypoxia has developed with subsequent increased VEGF production. The slower tumor growth rate after FS exposure may have prevented the development of tumor hypoxia, which could explain the decreased levels of VEGF in those tumors.

Another regulator of angiogenesis is estrogen. It has been shown that estrogen modulates angiogenesis, both under physiological and pathological conditions, mainly via effects on endothelial cells [33]. In the female reproductive tract, where angiogenesis is a normal physiologic event, as well as in endometrial and breast cancer, VEGF expression is regulated by sex steroids and an estrogen responsive element in the gene for VEGF has been identified [34,35]. In animal models and in vitro the mammalian lignans ED and EL, which are produced after FS diet, have been shown to inhibit aromatase and stimulate production of sex-hormone-binding globulin, which is hypothesized to lead to a reduction of endogenous estrogen levels and lengthening of the estrous cycle [36–38]. Moreover, it has been shown that EL and genistein inhibit angiogenesis in vitro by decreased proliferation and migration of endothelial cells and decreased levels of VEGF in tumors [39,40]. Thus, a diet of FS could affect angiogenesis and VEGF production both by direct cellular effects and indirectly by reducing the endogenous estrogen production.

In a previous study, we have shown that 10% FS can inhibit breast cancer growth and metastasis by downregulation of expression of insulin growth factor-1 and epidermal growth factor receptor [41]. The present study suggests that the slow tumor growth rate seen in the mice after a FS diet may also be attributed to its effect on tumor angiogenesis.

We conclude that diet supplementation with FS given to nude mice with established human breast cancer xenografts reduced the tumor growth rate and the development of distant metastasis. We also report decreased extracellular levels of VEGF in FS-exposed tumors, which may be one mechanistic explanation to both the reduced growth rate and the decreased incidence of metastasis. Further studies should focus on the in vivo effects of mammalian lignans on solid human tumors where microdialysis may prove to be a useful tool in providing data on molecular events of tumors in situ.

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References

[1] G.R. Howe, T. Hirohata, T.G. Hislop, J.M. Iscovich, J.M. Yuan, K. Katsouyanni, F. Lubin, E. Marubini, B. Modan, T. Rohan, Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies, J. Natl. Cancer Inst. 82 (1990) 561–569.

[2] D.P. Rose, A.P. Boyar, E.L. Wynder, International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption, Cancer 58 (1986) 2363–2371.

[3] M.J. Messina, V. Persky, K.D. Setchell, S. Barnes, Soy intake and cancer risk: a review of the in vitro and in vivo data, Nutr. Cancer 21 (1994) 113–131.

[4] L.U. Thompson, Experimental studies on lignans and cancer, Bailliere's Clin. Endocrinol. Metab. 12 (1998) 691–705.

[5] L.U. Thompson, S.E. Rickard, L.J. Orcheson, M.M. Seidl, Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis, Carcinogenesis 17 (1996) 1373–1376.

[6] J.C. Tou, L.U. Thompson, Exposure to flaxseed or its lignan component during different developmental stages influences rat mammary gland structures, Carcinogenesis 20 (1999) 1831–1835.

[7] W.E. Ward, F.O. Jiang, L.U. Thompson, Exposure to flaxseed or purified lignan during lactation influences rat mammary gland structures, Nutr. Cancer 37 (2000) 187–192.

[8] S.P. Borriello, K.D. Setchell, M. Axelson, A.M. Lawson, Production and metabolism of lignans by the human faecal flora, J. Appl. Bacteriol. 58 (1985) 37–43.

[9] L.U. Thompson, P. Robb, M. Serraino, F. Cheung, Mammalian lignan production from various foods, Nutr. Cancer 16 (1991) 43–52.

[10] G. Neufeld, T. Cohen, S. Gengrinovitch, Z. Poltorak, Vascular endothelial growth factor (VEGF) and its receptors, Fed. Am. Soc. Exp. Biol. J. 13 (1999) 9–22.

[11] J. Folkman, Angiogenesis in cancer, vascular, rheumatoid and other disease, Nat. Med. 1 (1995) 27–31.

[12] H. Yoshiji, D.E. Gomez, M. Shibuya, U.P. Thorgeirsson, Expression of vascular endothelial growth factor, its receptor, and other angiogenic factors in human breast cancer, Cancer Res. 56 (1996) 2013–2016.

[13] B. Linderholm, K. Grankvist, N. Wilking, M. Johansson, B. Tavelin, R. Henriksson, Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment, J. Clin. Oncol. 18 (2000) 1423–1431.

[14] B. Linderholm, B. Tavelin, K. Grankvist, R. Henriksson, Vascular endothelial growth factor is of high prognostic value in node-negative breast carcinoma, J. Clin. Oncol. 16 (1998) 3121–3128.

[15] H.T. Zhang, P.A. Scott, L. Morbidelli, S. Peak, J. Moore, H. Turley, A.L. Harris, M. Ziche, R. Bicknell, The 121 amino acid isoform of vascular endothelial growth factor is more strongly tumorigenic than other splice variants in vivo, Br. J. Cancer 83 (2000) 63–68.

[16] J.E. Price, R.D. Zhang, Studies of human breast cancer metastasis using nude mice, Cancer Metastasis Rev. 8 (1990) 285–297.

[17] D.P. Rose, J.M. Connolly, X.H. Liu, Effects of linoleic acid on the growth and metastasis of two human breast cancer cell lines in nude mice and the invasive capacity of these cell lines in vitro, Cancer Res. 54 (1994) 6557–6562.

[18] C.L. Meschter, J.M. Connolly, D.P. Rose, Influence of regional location of the inoculation site and dietary fat on the pathology of MDA-MB-435 human breast cancer cell-derived tumors grown in nude mice, Clin. Exp. Metastasis 10 (1992) 167–173.

[19] J.E. Price, A. Polyzos, R.D. Zhang, L.M. Daniels, Tumorigenicity and metastasis of human breast carcinoma cell lines in nude mice, Cancer Res. 50 (1990) 717–721.

[20] P.G. Reeves, F.H. Nielsen, G.C. Fahey Jr., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, J. Nutr. 123 (1993) 1939–1951.

[21] U. Ungerstedt, Microdialysis – principles and applications for studies in animals and man, J. Intern. Med. 230 (1991) 365–373.

[22] C. Dabrosin, K. Ollinger, U. Ungerstedt, M. Hammar, Variability of glutathione levels in normal breast tissue and subcutaneous fat during the menstrual cycle: an in vivo study with microdialysis technique, J. Clin. Endocrinol. Metab. 82 (1997) 1382–1384.

[23] N. Ferrara, T. Davis-Smyth, The biology of vascular endothelial growth factor, Endocr. Rev. 18 (1997) 4–25.

[24] E. Tischer, R. Mitchell, T. Hartman, M. Silva, D. Gospodarowicz, J.C. Fiddes, J.A. Abraham, The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing, J. Biol. Chem. 266 (1991) 11947–11954.

[25] K.A. Houck, D.W. Leung, A.M. Rowland, J. Winer, N. Ferrara, Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms, J. Biol. Chem. 267 (1992) 26031–26037.

[26] M. Toi, S. Kondo, H. Suzuki, Y. Yamamoto, K. Inada, T. Imazawa, T. Taniguchi, T. Tominaga, Quantitative analysis of vascular endothelial growth factor in primary breast cancer, Cancer 77 (1996) 1101–1106.

[27] A.H. Lee, E.A. Dublin, L.G. Bobrow, R. Poulsom, Invasive lobular and invasive ductal carcinoma of the breast show distinct patterns of vascular endothelial growth factor expression and angiogenesis, J. Pathol. 185 (1998) 394–401.

[28] M. Relf, S. LeJeune, P.A. Scott, S. Fox, K. Smith, R. Leek, A. Moghaddam, R. Whitehouse, R. Bicknell, A.L. Harris, Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis, Cancer Res. 57 (1997) 963–969.

[29] D.B. Rifkin, D. Moscatelli, J. Bizik, N. Quarto, F. Blei, P. Dennis, R. Flaumenhaft, P. Mignatti, Growth factor control of extracellular proteolysis, Cell Differ. Dev. 32 (1990) 313–318.

[30] H. Kimura, A. Weisz, T. Ogura, Y. Hitomi, Y. Kurashima, K. Hashimoto, F. D'Acquisto, M. Makuuchi, H. Esumi, Identification of hypoxia-inducible factor 1 ancillary sequence and its function in vascular endothelial growth factor gene induction by hypoxia and nitric oxide, J. Biol. Chem. 276 (2001) 2292–2298.

[31] S. Pal, K. Datta, D. Mukhopadhyay, Central role of p53 on regulation of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) expression in mammary carcinoma, Cancer Res. 61 (2001) 6952–6957.

[32] R. Bos, H. Zhong, C.F. Hanrahan, E.C. Mommers, G.L. Semenza, H.M. Pinedo, M.D. Abeloff, J.W. Simons, P.J. van Diest, E. van der Wall, Levels of hypoxia-inducible factor-1 alpha during breast carcinogenesis, J. Natl. Cancer Inst. 93 (2001) 309–314.

[33] D.W. Losordo, J.M. Isner, Estrogen and angiogenesis: a review, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 6–12.

[34] S.M. Hyder, Z. Nawaz, C. Chiappetta, G.M. Stancel, Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor, Cancer Res. 60 (2000) 3183–3190.

[35] S.M. Hyder, G.M. Stancel, Regulation of angiogenic growth factors in the female reproductive tract by estrogens and progestins, Mol. Endocrinol. 13 (1999) 806–811.

[36] M. Loukovaara, M. Carson, A. Palotie, H. Adlercreutz, Regulation of sex hormone-binding globulin production by isoflavonoids and patterns of isoflavonoid conjugation in HepG2 cell cultures, Steroids 60 (1995) 656–661.

[37] H. Adlercreutz, C. Bannwart, K. Wahala, T. Makela, G. Brunow, T. Hase, P.J. Arosemena, J.T. Kellis Jr., L.E. Vickery, Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens, J. Steroid Biochem. Mol. Biol. 44 (1993) 147–153.

[38] L.J. Orcheson, S.E. Rickard, M.M. Seidl, L.U. Thompson, Flaxseed and its mammalian lignan precursor cause a lengthening or cessation of estrous cycling in rats, Cancer Lett. 125 (1998) 69–76.

[39] T. Fotsis, M. Pepper, H. Adlercreutz, T. Hase, R. Montesano, L. Schweigerer, Genistein, a dietary ingested isoflavonoid, inhibits cell proliferation and in vitro angiogenesis, J. Nutr. 125 (1995) 790S–797S.

[40] Z.M. Shao, J. Wu, Z.Z. Shen, S.H. Barsky, Genistein exerts multiple suppressive effects on human breast carcinoma cells, Cancer Res. 58 (1998) 4851–4857.

[41] J. Chen, M. Stavro, L.U. Thompson, Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epithelial cell growth factor receptor, Nutr. Cancer (2002) in press.