Chapter 3
Beneficial Influence of Diets Enriched with Flaxseed and Flaxseed Oil on Cancer

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Abstract Dietary flaxseed and flaxseed oil are commonly consumed for their suggested anticancer effects. Flaxseed oil has an exceptionally high level of the omega-3 fatty acid α-linolenic acid and flaxseed is also the richest dietary source of phytoestrogens called lignans. This chapter provides information on flaxseed and flaxseed oil, including their composition and effects on the prevention and treatment of cancer. The major focus is on the effects in breast, colorectal and prostate cancer as observed in preclinical studies in cell culture and animal models, epidemiological and clinical studies. Limited studies on the effects in other forms of cancer are also discussed. Recent evidence supporting a potential anticancer role of flaxseed and flaxseed oil is for breast cancer. Extensive studies in rodent models suggest that flaxseed and its oil can reduce the various stages of carcinogenesis and there is increasing support from epidemiological and clinical studies. Studies in rodent models also suggest that flaxseed and its oil do not interfere with and may rather enhance the action of breast cancer drugs, including tamoxifen and trastuzumab. Regarding colorectal and prostate cancer, there are fewer studies with less consistent results. However, a protective effect is shown in general studies. The research in other forms of cancer is limited, inconsistent and warrants further investigation. Potential mechanisms of the action of flaxseed oil including effects on the properties of the cell membrane, the regulation of transcription, lipid peroxidation and others are discussed. Safety of diets enriched in flaxseed and flaxseed oil and flaxseed's regulatory status are outlined. Current limitations in the research and future directions are provided.

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3.1 Introduction

Cancer is one of the leading causes of death in the Western world (World Cancer Research Fund/American Institute for Cancer Research 2007). In North America, colorectal, prostate and breast are three of the most common and deadliest forms of cancer (Canadian Cancer Society 2010; American Cancer Society 2011). Although improvements in screening, detection and treatment have been made, strategies for prevention and further improvement of treatment outcomes are being sought. The role of bioactive dietary components in preventing and treating cancer has been a research focus for many years. Many cancer patients look to complementary and alternative medicine (CAM) to prevent cancer and assist traditional cancer therapy. A number of dietary compounds have been recommended for cancer treatment or prevention and are readily available to the general public through published books and websites. Studies have shown that flaxseed and flaxseed oil are among the most commonly used dietary supplements among cancer patients in North America (Boon et al. 2007; Greenlee et al. 2009; Rausch et al. 2010; Anderson and Taylor 2012; Boucher et al. 2012). Thus, there is a need to understand the scientific support for these agents.

This chapter will review the evidence for the role of flaxseed oil and its source, flaxseed, in the prevention and treatment of breast, colorectal and prostate cancer. Limited studies on the effect of flaxseed and its oil in other cancer types will also be discussed. Results from preclinical studies in rodent models and cell culture experiments, as well as observational and experimental human studies will be described and summarized. Potential mechanisms of effect and safety aspects of flaxseed and flaxseed oil rich diets will be discussed. Finally, comments on the limitations, current research gaps and future directions in the use of flaxseed oil-rich diets will be provided.

3.2 Flaxseed and Flaxseed Oil Composition

Of interest and the main reason for the use of flaxseed as a dietary supplement for health benefits is its high amount of oil, rich in the omega-3 (n-3) polyunsaturated fatty acid (PUFA) α-linolenic acid (ALA), the high amount of dietary fiber, high quality protein, and phytoestrogens called lignans. While flaxseed's exact composition varies by growth location, cultivar, and environment, it typically contains approximately 30 % dietary fiber, 20 % protein, 40 % oil and 820–1,050 μmol lignan per 100 g of flaxseed (Daun et al. 2003; Liu et al. 2006; Thompson et al. 2006).

Flaxseed oil's effect in reducing cancer growth has been of growing interest in recent years. The approximate composition of flaxseed oil is shown in Table 3.1 (Daun et al. 2003). Flaxseed oil is comprised primarily of neutral lipids (acylglycerols and fatty acids) and some polar lipids (glycolipids and phospholipids). About 57 % of flaxseed oil is ALA, an essential fatty acid, which

Table 3.1 Approximate fatty acid composition of flaxseed oil

Fatty acid class	% of total fat
Saturates	9.0
Monounsaturates	18.0
Polyunsaturates	73.0
Linoleic acid	16.0
α-linolenic acid	57.0

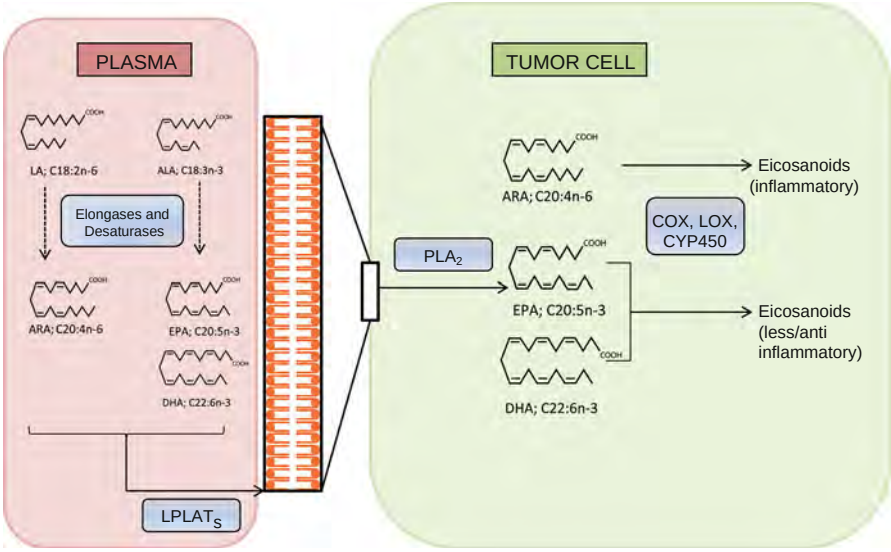

Fig. 3.1 n-3 and n-6 fatty acid metabolism. ALA and LA, the parent n-3 and n-6 fatty acids are converted by elongase and desaturase enzymes into the long chain fatty acids ARA (n-6), EPA (n-3) and DHA (n-3). Free PUFA are esterified into the membrane phospholipids by LPLATs and can then be released to the intracellular pool by PLA2. Free ARA, EPA and DHA are converted into eicosanoids through the actions of COX, LOX and CYP450 enzymes. n-3 and n-6 derived eicosanoids have different biological effects. *ALA* α-linolenic acid, *ARA* arachidonic acid, *COX* cyclooxygenases, *CYP450* cytochrome P450, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *LA* linoleic acid, *LOX* lipoxygenases, *LPLATS* lysophospholipid acyltransferases, *PLA2* phospholipase A2

can be metabolized to a limited extent to the longer chain n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), through a series of elongation (addition of 2 carbon) and desaturation (double bond insertion) steps (Cunnane 2003; Hall et al. 2006) (Fig. 3.1). Flaxseed is the richest plant source of ALA. Other sources rich in ALA include the chia seed, walnuts, perilla, canola, soybean and their oils.

This chapter focuses primarily on the specific effects of flaxseed oil as well as its source, flaxseed, on breast, colorectal and prostate carcinogenesis. It is important to note, however, that effects observed in intervention studies using flaxseed may not necessarily be solely due to the oil but also to other components including its lignan, fiber and protein.

3.3 Flaxseed and Cancer

The majority of the research focusing on the effect of flaxseed and flaxseed oil in cancer has been in hormone-related cancers, especially breast cancer. This came about because initial interest on the effect of flaxseed in cancer relates to its high amount of lignan. The predominant lignan in flaxseed is secoisolariciresinol diglucoside (SDG), which can be metabolized by colonic microbiota to the mammalian lignans enterodiol and enterolactone (Thompson et al. 1991, 2006). These mammalian lignans have chemical structural similarity to 17β-estradiol (E2), thus they are thought to have antiestrogenic/estrogenic properties that may influence hormone-related diseases, such as breast cancer (Adlercreutz 2007).

Elevated exposure to E2 is known to modulate breast cancer risk and factors that increase lifetime exposure to E2 such as early menarche, late menopause, hormone therapy use and adiposity are independent risk factors for breast cancer. As the research in the field of flaxseed and breast cancer progressed, the focus on the effect of the whole seed moved on to the study of the effect of its individual components including flaxseed oil and lignans. Through this research, support for the potential anticancer role of ALA-rich flaxseed oil developed. Unlike lignans, the proposed mechanisms of flaxseed oil effect do not relate directly to hormonal effects. Therefore, while ALA-rich flaxseed oil has mainly been studied in hormone-related cancers, effects have been observed in other types of cancer as well.

A number of preclinical models outlined in Fig. 3.2 have been useful in elucidating the effects of dietary components in carcinogenesis. These include rodent and cell culture models which have been used to study the effect of dietary agents such as flaxseed and flaxseed oil from a prevention or treatment perspective.

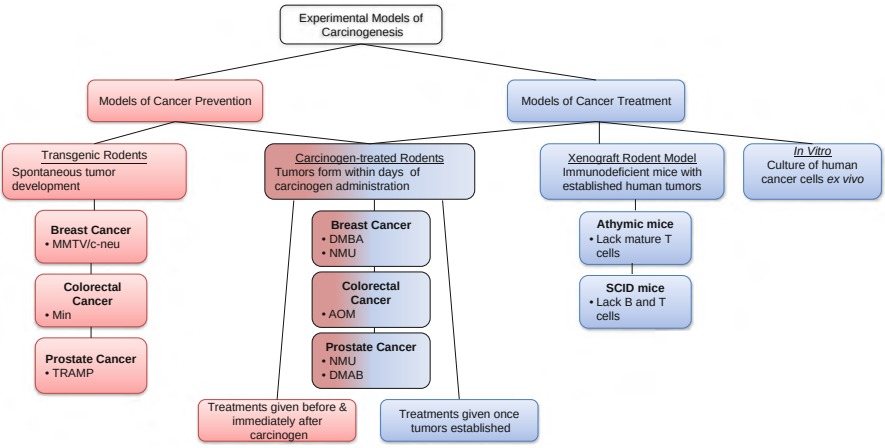

Fig. 3.2 Experimental models of carcinogenesis. *AOM* azoxymethane, *DMAB* 3,2′-dimethyl-4-aminobiphenyl, *DMBA* dimethylbenz(α)anthracene, *MMTV* mouse mammary tumor virus, *NMU* *N*-nitrosomethyl-urea, *SCID* severe combined immunodeficiency, *TRAMP* transgenic adenocarcinoma of the mouse prostate

3.4 Studies of Flaxseed and Flaxseed Oil in Breast Cancer

Breast cancer is a heterogeneous disease in terms of invasiveness, initiation site (duct or lobule), as well as cell receptor expression. Important protein receptors that can modify growth, prognosis and treatment are the estrogen receptor (ER) and the human epidermal growth factor receptor-2 (HER2). Based on these receptors, breast cancers are divided into molecular subtypes which have different prognosis and treatment approaches (Carey et al. 2006; Yang et al. 2007). When investigating potential chemopreventative or treatment options for breast cancer such as flaxseed, the specific subtypes and receptor status should be considered.

3.4.1 Breast Cancer Prevention

3.4.1.1 Preclinical Studies

The carcinogen-induced rodent model has been a useful tool in elucidating the effect of flaxseed and its components in the prevention of breast cancer (Table 3.2). The effect of flaxseed in breast cancer was first evaluated using rat models of the various stages of carcinogenesis (pre-initiation, initiation and promotion). The first study looked at the effects of diets rich in flaxseed on early markers of mammary carcinogenesis. Sprague-Dawley rats were fed high fat diets containing either no flaxseed (control), 5 or 10 % whole ground flaxseed (FF) or 5 or 10 % defatted flaxseed meal (FM) all matched for macronutrient and caloric content. The FF diets contributed 1.9–3.8 % flaxseed oil whereas the FM diets contributed 0.14–0.28 % flaxseed oil. Diets were fed for 4 weeks and measures of mitotic index, cell proliferation and nuclear aberrations after administration of the carcinogen dimethylbenz(α)anthracene (DMBA) in various structures of the mammary gland were conducted. The mitotic index in the terminal end buds (TEB), structures thought to be most highly implicated in carcinogenesis, was significantly lower in rats fed the 5 and 10 % FF diets with no significant reduction in those fed the FM diet. Similarly, cell proliferation and nuclear aberrations were significantly lower only in the TEB of rats fed the 5 % FF diet. The greater effect observed in the FF group which contains flaxseed oil suggests that the oil is likely playing an important role in flaxseed's anticancer effect at the pre-initiation stages (Serraino and Thompson 1991). In a follow up study by Serraino and Thompson (1992a), the effect of a 5 % FF diet on DMBA-induced carcinogenesis was shown to be complex. Rats were treated with DMBA at 50 days of age and followed for 21 weeks. The FF diet was fed either (i) throughout the study period (initiation and promotion), (ii) for 4 weeks prior to DMBA and followed by feeding with the control diet (initiation only) or (iii) starting 1 week after DMBA administration (promotion). Interestingly, when looking at the effect of flaxseed feeding throughout the study period (initiation and promotion) there was no difference in tumor

Table 3.2 Summary of studies investigating effect of flaxseed, flaxseed oil and ALA on breast cancer

Model	Treatments/measures	Results	References
In vitro studies			
MCF-7 cells	50 μM ALA + 1nM E2 for 5 days	↓ cell proliferation by 33 %	Truan et al. (2010)
MCF-7 cells	Up to 100 μM ALA for 24, 48, 72 h	↓ cell growth dose and time dependently ↑ apoptosis dose dependently	Kim et al. (2009)
MCF-7, MDA MB 231 cells	71.83 μM ALA, 5 days	↓ cell growth in MDA MB 231 but not MCF-7 No effect on cell viability	Chajes et al. (1995)
MDA MB 231	10–200 μM ALA, 24 h	↓ cell number	Horia and Watkins (2005)
SKBr3, BT 474 cells	10–20 μM ALA ± trastuzumab, 48 h	↓ HER2 expression dose dependently ↓ cell proliferation when ALA combined with trastuzumab	Menendez et al. (2006)
In vivo animal studies			
OVX athymic mice with MCF-7 xenografts	BD, FSO (38.5 g/kg), SDG (1 g/kg) and FSO + SDG Low E2	↑ tumor regression rate in all groups *vs.* control ↓ cell proliferation in all groups compared to control No effect on apoptosis	Saggar et al. (2010b)
OVX athymic mice with MCF-7 xenografts	BD, 10 % FS Low E2	↓ tumor growth, cell proliferation and ↑ apoptosis in FS *vs.* control	Chen et al. (2009)
OVX athymic mice with MCF-7 xenografts	BD, 10 % FS diet Low E2	No difference in tumor area, cell proliferation or apoptosis in FS *vs.* control	Power et al. (2008); Saarinen et al. (2006)
OVX athymic mice with MCF-7 xenografts	BD, 4 % FSO High E2	↓ tumor growth, cell proliferation and ↑ apoptosis in FSO *vs.* control	Truan et al. (2010)
OVX athymic mice with MCF-7 xenografts	BD, ED (15 mg/kg), EL (15 mg/kg) or 10 % FS High E2	↓ tumor growth and angiogenesis in all treatments *vs.* control	Bergman Jungestrom et al. (2007)

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
Athymic mice with MDA-MB-435 xenografts	BD, 10 % FS, SDG and FSO at levels present in 10 % FS or SDG + FSO High E2	↓ tumor growth, cell proliferation and ↑ apoptosis in all treatments except SDG *vs.* control	Wang et al. (2005)
Athymic mice with MDA-MB-435 xenografts	BD, 10 % FS High E2	↓ tumor growth and cell proliferation in FS compared to control	Chen et al. (2002)
Sprague-Dawley rats with DMBA-induced tumors (progression and tumor development stages)	BD, 2.5 or 5 % FS diet or FSO or SDG at levels present in 5 % FS	↓ established tumor growth in 2.5 and 5 % FS and FO compared to control; no effect of SDG	Thompson et al. (1996)
	Diet treatment started 13 weeks post DMBA	↓ new tumor volume in SDG *vs.* control; no effect of 2.5 or 5 % FS or FO No difference in tumor incidence and number between groups	
Sprague-Dawley rats with DMBA-induced tumors (initiation and early promotion stages)	BD, 5 % FS diet	↓ tumor size in rats fed FS at promotional stage; no effect of FS fed at initiation	Serraino and Thompson (1992a)
	FS fed at (i) initiation, (ii) early promotion or (iii) initiation and promotion	↑ tumor burden in promotion only *vs.* initiation and promotion FS groups	
Sprague-Dawley rats with DMBA-induced (initiation stage)	BD, 5 or 10 % FS flour (FF; 1.9–3.8 % FO) or defatted FS meal (FM; 0.14–0.28 % FO)	↓ mitotic index in terminal end buds of 5 % and 10 % FF groups	Serraino and Thompson (1991)
	Diets fed for 4 weeks pre DMBA exposure and rats sacrificed 24 h post DMBA	↓ cell proliferation in terminal end buds of 5 % FF groups ↓ nuclear aberrations in terminal end buds of 5 % FF, in terminal duct of 5 and 10 % FM, in alveolar buds of 10 % FF and 10 % FM	
Sprague-Dawley rats with NMU-induced tumors (early promotion stage)	BD, 2.5 or 5 % FS	↓ tumor invasiveness and grade in 2.5 and 5 % FS *vs.* control	Rickard et al. (1999)
	Diet treatment started 2 days post NMU	No effects on final tumor weight, volume, multiplicity and incidence	

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
Sprague-Dawley rats with NMU-induced (initiation stage)	Diets contained either 15 % FSO or 15 % palm oil/sunflower oil	↑ tumor growth FSO + vit E compared to FSO – vit E; no difference in tumor area and multiplicity, latency or incidence	Cognault et al. (2000)
	FSO ± vit E and + vit E + oxidant	↓ tumor area, multiplicity, incidence and number in FSO + vit E + oxidant compared to FSO + vit E	
Tg.NK (MMTV-c-neu) model	BD, FS diets (0.006, 0.018, 0.054 %) starting at day 25	↓ tumor incidence, number of tumors per mouse and number of large tumors in 0.054 % FS group *vs.* control	Birkved et al. (2011)
		No effect on the number of tumor bearing mice and tumor multiplicity	
Tg.NK (MMTV-c-neu) model	Gavage of FSO or melatonin in corn oil starting at 4 weeks of age	No significant effect of FSO on tumor incidence, multiplicity	Rao et al. (2000)
	Varying dose of FSO	Trend toward ↓ number of tumors/mouse in high dose FSO	
		↓ weight of tumors/mouse and mean tumor weight in high dose FSO group	
Athymic mice with 410 and 410.4 xenografts	BD, FSO or 4:1 fish oil (FO):corn oil (CO) fed (i) before implantation, (ii) before implantation with removal of primary tumor, (iii) after implantation	(i) No difference in tumor incidence or tumor size	Fritsche and Johnson (1990)
		(ii) Primary tumors grew faster and were larger in the FSO group *vs.* CO	
		(iii) Primary tumors were smallest in the FSO group *vs.* FO and lowest metastasis in FSO	

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
In vivo animal studies: drug-diet interaction			
OVX athymic mice with BT-474 xenografts	TRAS ± FSO (80 g/kg)	↓ tumor area, cell proliferation and ↑ apoptosis in FSO + TRAS2.5 *vs.* TRAS2.5	Mason et al. (2010)
OVX athymic mice with MCF-7 xenografts	BD, FSO (38.5 g/kg), SDG (1 g/kg) and FO + SDG ± TAM Low E2	↓ tumor growth, cell proliferation and ↑ apoptosis in all treatment groups *vs.* control FSO and FSO + SDG had the greatest effects	Saggar et al. (2010a)
OVX athymic mice with MCF-7 xenografts	BD ± TAM, ± 5, 10 % FS Low E2	↓ tumor regrowth, cell proliferation and ↑ apoptosis in TAM + 10 % FS *vs.* TAM alone	Chen et al. (2007b)
OVX athymic mice with MCF-7 xenografts	BD ± TAM, ± 5, 10 % FS High E2	↓ tumor growth, cell proliferation and ↑ apoptosis in all groups *vs.* control 10 % FS as effective as TAM alone; TAM + 5 % FS more effective than TAM or 5 % alone in ↓ tumor growth	Chen et al. (2007a)
OVX athymic mice with MCF-7 xenografts	BD ± TAM, ± 10 % FS Low and high E2	Low E2: ↓ tumor growth, cell proliferation and ↑ apoptosis in FS and FS + TAM *vs.* TAM and control High E2: ↓ tumor growth, cell proliferation and ↑ apoptosis in all treatments *vs.* control; ↓ cell proliferation in FS + TAM *vs.* TAM alone	Chen et al. (2004)
Clinical and epidemiological studies			
Case control; 123 breast cancer patients, 59 controls	Fatty acid composition of breast adipose tissue	↓ breast cancer risk with increasing ALA levels in breast adipose tissue (p trend = 0.026)	Klein et al. (2000)

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Table 3.2 (continued)

Model	Treatments/measures	Results	References
Case control ; 365 breast cancer patients, 397 controls	Questionnaire and FFQ	↑ breast cancer risk with ALA intake, OR = 3.8 (1.5–9.4)	De Stefani et al. (1998)
Case control; 241 patients and 88 controls	Fatty acid composition of breast adipose tissue	↓ breast cancer risk with ALA breast adipose levels, adjusted OR = 0.39 (0.19–0.78), *p* trend = 0.01	Maillard et al. (2002)
Case control; 414 cases, 429 controls	FFQ for ALA intake	No association with breast cancer risk and ALA intake, OR = 1.27 (0.85–1.89), *p* trend = 0.284	Nkondjock et al. (2003a, b)
Case control; 196 cases, 388 controls	Fatty acid composition of serum phospholipids	No association with breast cancer risk and ALA levels in serum phospholipids OR = 1.36 (0.63–2.96), *p* trend = 0.424	Chajes et al. (1999)
Case Control; 322 cases, 1,030 controls	Erythrocyte fatty acid concentrations	No association with breast cancer risk and ALA levels of erythrocytes, OR = 0.99 (0.54–1.82), *p* trend = 0.59	Shannon et al. (2007)
Case Control; 103 cases, 309 controls	Erythrocyte fatty acid concentrations Dietary record	No association with breast cancer risk and ALA intake or erythrocyte composition	Kuriki et al. (2007)
Prospective Cohort; 121 breast cancer patients	Fatty acid methyl esters of breast adipose tissue	↓ breast cancer metastases when breast adipose ALA above 0.38 % of total fatty acids	Bougnoux et al. (1994)
Prospective cohort in 56,007 French women	Diet history questionnaires Followed for 8 years	↓ breast cancer hazard ratio with ALA intake from fruits and vegetables, and vegetable oils (*p* trend <0.0001, 0.017) ↑ with ALA intake from nut mixes (*p* trend 0.004) and processed foods (*p* trend 0.068)	Thiebaut et al. (2009)

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Table 3.2 (continued)

Model	Treatments/measures	Results	References
Cohort study; 62,573 women	FFQ for ALA intake	↓ breast cancer risk with ALA intake RR = 0.70 (0.51–0.97), p trend = 0.006	Voorrips et al. (2002)
Meta-analysis; fatty acid composition of adipose tissue/serum	Three cohort and seven case-control studies	Case control studies: high ALA content ↓ risk of breast cancer Cohort studies: no association between ALA content and breast cancer risk; in postmenopausal women ALA content ↑ breast cancer risk, RR = 1.14 (1.03–1.26)	Saadatian-Elahi et al. (2004)
RCT; 32 postmenopausal breast cancer patients	25 g FS muffin/day or control placebo muffin Biopsy tissue at diagnosis and surgery	↓ cell proliferation 34.2 % (p = 0.001) in FS group ↑ apoptosis 30.7 % (p = 0.007) in FS group ↓ HER2 expression 71 % (p = 0.003) in FS group	Thompson et al. (2005)

ALA α-linolenic acid, *BD* basal diet, *CO* corn oil, *DMBA* dimethylbenz(α)anthracene, *E2* estrogen, *ED* enterodiol, *EL* enterolactone, *FFQ* food frequency questionnaire, *FO* fish oil, *FS* flaxseed, *FSO* flaxseed oil, *HER2* human epidermal growth factor receptor 2, *NMU* *N*-nitrosomethyl-urea, *OR* odds ratio, *OVX* ovariectomized, *RCT* randomized controlled trial, *RR* relative risk, *SDG* secoisolariciresinol diglucoside, *TAM* tamoxifen, *TRAS* trastuzumab

burden (# of tumors/tumor bearing rat) or tumor volume compared to control. Dietary flaxseed fed only at the initiation stage tended to reduce the tumor burden, however, did not affect the final tumor size. On the other hand, flaxseed fed at the early promotion stage resulted in significantly smaller tumors compared to control despite greater tumor burden compared to the group fed the flaxseed diet throughout (Serraino and Thompson 1992a). Using the *N*-nitrosomethyl-urea (NMU)-induced rat model, Rickard et al. (1999), showed that dietary flaxseed fed 2 days post carcinogen administration had no effect on final tumor weight, volume, multiplicity or incidence although it did reduce the invasiveness and grade suggesting a flaxseed effect at the more advanced stages of carcinogenesis. The authors noted that the discrepancy in the results between their study and the previous DMBA-induced models described above may be due to a number of differences in experimental design including the use of soybean oil as the fat source in the basal diet (BD) which contributes ALA as opposed to the previously used corn oil-based BD which has very low ALA. Although the results of these studies were not straightforward, they stimulated a great interest in the role of dietary flaxseed in carcinogenesis.

Fewer studies have looked specifically at the role of flaxseed oil in the prevention of mammary carcinogenesis (Table 3.2). An early study compared the effect of various oils on mammary tumor growth in the C3H/Heston mouse model with DMBA administration and found flaxseed oil and fish oil fed mice had the lowest tumor incidence while corn oil and safflower oil had the greatest tumor incidence (Cameron et al. 1989). In a series of experiments, Cognault et al. (2000) demonstrated that flaxseed oil's effect on NMU-induced mammary tumor growth varies based on the presence of anti- or pro-oxidants in the diet, as (i) a flaxseed oil diet combined with vitamin E increased tumor growth in mice compared to a vitamin E-free diet, and (ii) a flaxseed oil diet with vitamin E plus prooxidant decreased tumor growth compared to the flaxseed oil with vitamin E diet alone. This study provides insight into how flaxseed oil may affect tumor growth (i.e. oxidation), but other dietary oils were not used so comparisons between flaxseed oil and other sources cannot be made (Cognault et al. 2000).

Flaxseed's effect in the prevention of HER2 overexpressing breast cancer has been studied using the MMTV/c-neu transgenic mouse model which spontaneously develops HER2+ tumors. When increasing levels of flaxseed were fed for 23 weeks, only the highest level of flaxseed (0.054 %) reduced tumor incidence, burden and number of large tumors compared to control levels. None of the flaxseed diets affected tumor multiplicity and number of tumor-bearing mice compared to control (Birkved et al. 2011). The levels used in this study were very low, almost 100 fold lower than the previously outlined studies, and therefore it is possible that a greater effect would have been achieved with higher levels of flaxseed in the diet. The effect of flaxseed oil has also been studied using this model. Mice were gavaged with 0.2 ml of oil containing increased proportion of flaxseed oil mixed into corn oil (0.05, 0.1 and 0.2 ml of flaxseed oil) for 30 weeks. The effect of flaxseed oil on mammary tumor development was complex; low dose of flaxseed oil resulted in a non significant increase in tumor incidence and number of tumors per mouse while there was a trend toward reduced tumor incidence with higher dose of flaxseed oil. The high dose flaxseed oil treated mice had lower overall weight of tumors per mouse and mean tumor weight compared to control (Rao et al. 2000). These results suggest that the n-6:n-3 ratio plays an important role in mediating the effect of flaxseed oil on HER2+ mammary tumorigenesis.

3.4.1.2 Clinical and Epidemiological Studies

Epidemiological and limited clinical studies that investigated flaxseed, flaxseed oil, ALA and breast cancer risk have produced inconsistent results (Table 3.2). A recent case-control study found that flaxseed consumption measured through Food Frequency Questionnaires (FFQ) significantly reduced breast cancer risk [OR = 0.82 (0.69–0.97)] (Lowcock et al. 2013). Two case control studies showed that ALA content in breast adipose tissue was inversely associated with breast cancer risk (Klein et al. 2000; Maillard et al. 2002). As well, a meta-analysis of five case-control studies found that there was a significant decrease in breast cancer risk with increasing levels of biomarkers of ALA intake (Saadatian-Elahi et al. 2004).

Contrary to these studies, a case control study in Uruguay found that ALA consumption measured by FFQs increased breast cancer risk (De Stefani et al. 1998), which may be partially explained by the high intake of red meat in Uruguay accounting for a large proportion of the ALA intake rather than vegetable sources (Bougnoux and Chajes 2003). Other case control studies measuring ALA intake with FFQs and erythrocyte ALA content all found that there was no association between ALA and breast cancer risk (Chajes et al. 1999; Nkondjock et al. 2003a; Kuriki et al. 2007; Shannon et al. 2007).

Cohort studies show more promise in terms of the potential role of ALA in breast cancer prevention. One study found that the ALA content in the breast adipose tissue was inversely associated with risk of subsequent metastasis in 121 non-metastatic breast carcinoma patients. When ALA levels were above 0.38 % of breast fat, there was a five-fold reduction in risk (Bougnoux et al. 1994). Similarly, a cohort study in the Netherlands measured ALA intake with a validated FFQ and found that intake was inversely associated with breast cancer risk (Voorrips et al. 2002). The food source of ALA may play an integral part in its effectiveness as a breast cancer preventative compound as highlighted in a French cohort study (Thiebaut et al. 2009). ALA intake from fruits and vegetables as well as vegetable oils as measured by a FFQ was inversely related to breast cancer risk whereas ALA from nuts and processed foods increased risk. Menopausal status may also alter the effects of ALA as a meta-analysis of three cohort studies found that in postmenopausal women only, ALA as measured by biomarkers increased breast cancer risk (Saadatian-Elahi et al. 2004). There are several limitations associated with the studies above which may explain inconsistent findings including variation in biomarkers used for ALA intake, poor FFQs, population characteristics (menopausal status, cancer subtype, BMI), quartiles of intake used, and the food source. To our knowledge there have been no completed clinical trials that specifically studied flaxseed or its components on breast cancer prevention. One ongoing study that will hopefully provide a clearer picture of flaxseed's role in preventing breast cancer is investigating the effect of a flaxseed enriched diet for 6 months on biomarkers of breast cancer (proliferation, apoptosis, and estrogen receptor genes) in premenopausal women at high risk of developing breast cancer (NCT00794989).

3.4.2 Breast Cancer Treatment

3.4.2.1 Preclinical Studies

In vitro studies are useful for investigating the role of potential anticancer agents as they provide information on specific effects and mechanisms of action to build upon in future *in vivo* and clinical studies. To date, *in vitro* studies investigating the role of ALA on breast cancer cell lines have produced inconsistent results which seem dependent on the receptor expression of the cells and ALA dose, as well as environmental factors such as estrogen levels (Table 3.2). Some studies in the ER+, low HER2 MCF-7 breast cancer cell line showed promise for ALA to inhibit

growth as (i) cell proliferation was reduced by 33 % when cells were treated with 50 μM ALA in a high estrogen environment (1 nM) for 5 days (Truan et al. 2010), and (ii) ALA concentrations varying from 0 to 100 μM for 24–72 h dose and time dependently inhibited MCF-7 cell growth and induced apoptosis (Kim et al. 2009). However, one study did find that 71.83 μM ALA for 5 days did not reduce MCF-7 cell growth (Chajes et al. 1995). In the HER2 overexpressing cell lines BT-474 and SKBr3, 10–20 μM ALA for 48 h dose dependently decreased HER2 expression at the transcriptional level, which would lead to a decrease in cancer cell growth through a reduction in growth factor signaling (Menendez et al. 2006). Finally, two studies of ALA effect in the basal MDA MB 231 cell lines (ER−, low HER2) found that 10–200 μM ALA treatment for time ranging from 24 h to 5 days decreased cell proliferation and growth (Chajes et al. 1995; Horia and Watkins 2005). Overall *in vitro* studies indicate ALA likely decreases breast cancer cell growth, but further exploration into the specific cell lines affected, adequate doses and mechanism is needed.

Rodent models with established tumors are often used to investigate the effect of dietary components in the treatment of breast cancer, and early studies with these models suggested a reduction in growth of established tumors with flaxseed and flaxseed oil supplementation (Table 3.2). For example, Sprague-Dawley rats were fed a BD or a 1.82 % flaxseed oil diet, BD with SDG treatment or 5 or 10 % flaxseed diets starting 13 weeks post DMBA administration when mammary tumors were established (Thompson et al. 1996). At the end of the study, tumors that were established at the start of treatment regressed in all treatments. All treatments except flaxseed oil reduced total tumor volume (established and new) compared to control, but only SDG lowered new tumor volume suggesting that the lignans are more effective at inhibiting new tumor development whereas flaxseed oil is more effective at reducing the growth of established tumors. An early study compared the effect of corn oil, flaxseed oil and fish oil diets on the growth of implanted tumors derived from mouse mammary tumors (410 and 410.1) (Fritsche and Johnston 1990). There was no difference between the diets on the growth of 410 tumors; however, flaxseed oil had the greatest effect at reducing the growth and metastasis of 410.1 tumors. These results further support the potential of flaxseed oil in reducing the growth of established mammary tumors.

Flaxseed, flaxseed oil and lignan effects have been studied in the xenograft model with various human breast cancer cell lines and at low and high circulating levels of E2. The effects vary depending on experimental conditions suggesting that the effect may differ based on cancer subtype and menopausal stage. Three studies observed the effect of dietary flaxseed and its components on established ER negative (ER−) MDA-MB-435 tumor growth (Chen et al. 2002; Dabrosin et al. 2002; Wang et al. 2005). A 10 % flaxseed diet fed for 7 weeks reduced the palpable tumor growth, lymph node metastasis, tumor cell proliferation and the expression of the marker of angiogenesis, VEGF (Chen et al. 2002; Dabrosin et al. 2002). Established MDA-MB-435 tumors were shown to have significantly lower growth rate, cell proliferation and increased apoptosis compared to control in athymic mice fed either 10 % flaxseed or 4 % flaxseed oil diets both alone and when combined with SDG treatment while SDG alone did not affect the palpable tumor growth rate (Wang et al. 2005). These results suggest that the oil may be the most effective component at reducing ER− tumor growth.

The role of flaxseed and its components in modulating the growth of MCF-7 (ER+, low HER2) breast tumors was the focus of several studies. At high circulating E2 levels, 5 and 10 % flaxseed diets were both shown to reduce the growth of established MCF-7 human breast tumors in the athymic mouse model which was related to a reduction in cell proliferation and increase in apoptosis (Chen et al. 2007b). Established MCF-7 tumors regressed upon removal of the E2 pellet (negative control) to lower circulating E2 levels but the regression caused by flaxseed did not differ from that of the control (Saarinen et al. 2006; Chen et al. 2007a). At low circulating E2 levels, both flaxseed oil and SDG reduced the growth of MCF-7 xenografts in athymic mice although SDG had the greatest effect (Saggar et al. 2010a). The effect on tumor growth was related to changes in cell proliferation rather than apoptosis. Flaxseed oil's anti-tumorigenic effect was supported by a study which showed that the flaxseed cotyledon, rich in flaxseed oil but low in lignan, similarly reduces the growth of MCF-7 tumors (Chen et al. 2011). At high circulating levels of E2, dietary flaxseed oil significantly reduced MCF-7 tumor growth rate in athymic mice compared to control (Truan et al. 2010). In contrast, in a similarly designed study, dietary SDG did not affect MCF-7 tumor growth rate compared to control (Truan et al. 2012). Evidently, the effect of flaxseed and its components on the growth of ER+ xenografts depends on the estrogen environment; however, flaxseed oil was shown to reduce tumor growth at both low and high circulating levels of E2.

3.4.2.2 Diet-Drug Interactions

Studies have investigated the potential interaction of flaxseed and its components with tamoxifen (TAM), a primary adjuvant therapy for the treatment of ER+ breast cancer (Table 3.2). At high circulating levels of E2, flaxseed enhanced the tumor-suppressing effect of TAM in the athymic mouse model with MCF-7 xenografts (Chen et al. 2004, 2007a, b). Additionally, at low circulating levels of E2, 10 % flaxseed prevented the tumor regrowth seen with TAM treatment alone through a decrease in cell proliferation and an increase in apoptosis (Chen et al. 2004, 2007a). Saggar et al. (2010a, b) investigated the effect of flaxseed oil, SDG and flaxseed oil + SDG in combination with TAM treatment in athymic mice with established MCF-7 human breast tumors. An E2 pellet was implanted into the mice to stimulate tumor growth. On removal, circulating E2 levels fall to within the range seen in postmenopausal women and MCF-7 tumors (E2 dependent) regressed. The tumors regressed to a smaller size in all of the treatment groups compared to control with the greatest effect seen in mice fed the flaxseed oil diet. Similarly, all treatments reduced cell proliferation and increased apoptosis with the greatest effect seen in mice fed the flaxseed oil diet (Saggar et al. 2010b). The effect of flaxseed oil in enhancing TAM action is supported by the results of a study which showed that a diet enriched with the flaxseed cotyledon fraction, rich in flaxseed oil and low in lignans, similarly showed a reduction in tumor growth rate alone and when combined with TAM while TAM alone did not reduce tumor growth (Chen et al. 2011).

Flaxseed oil has also been studied for its interaction with trastuzumab (TRAS, Herceptin), a primary therapy used in the treatment of HER2 overexpressing breast cancer (Menendez et al. 2006; Mason et al. 2010). In the athymic mouse model, 8 % dietary flaxseed oil was shown to enhance the effectiveness of TRAS (2.5 mg/kg) in reducing the growth of BT-474 xenografts (HER2+, ER+). This effect was related to both increased apoptosis and reduced cell proliferation (Mason et al. 2010). *In vitro* work has also demonstrated a significant synergism between ALA and TRAS. Treating BT-474 cells with both ALA (2.5–40 μM) and TRAS (5 μg/ml) resulted in greater cytotoxicity compared to TRAS alone (Menendez et al. 2006).

Although there are promising preclinical results supporting dietary flaxseed and flaxseed oil as complementary agents along with TAM and TRAS treatment, the effect must be confirmed in humans before any recommendations can be made regarding their therapeutic applications.

3.4.2.3 Clinical Studies

Very few studies have investigated flaxseed, flaxseed oil or ALA as a complementary breast cancer treatment in humans (Table 3.2). One randomized placebo controlled double blind study determined the effect of 25 g/day flaxseed incorporated into a muffin on postmenopausal women with newly diagnosed breast cancer (Thompson et al. 2005). In the flaxseed group, cell proliferation and HER2 expression decreased by 34 and 71 % respectively, and apoptosis increased by 31 %, compared to baseline while there were no changes in the placebo control group. An ongoing double blind, placebo controlled randomized control trial is investigating the effect of a 25 g/day flaxseed supplement with and without the aromatase inhibitor drug anastrozole in postmenopausal, ER + breast cancer patients with primary analysis on changes in proliferation, apoptosis and receptor expression (NCT00612560).

3.5 Studies of Flaxseed and Flaxseed Oil on Colorectal Cancer

Fewer studies have investigated the role of flaxseed and its components on colorectal carcinogenesis compared to breast/mammary carcinogenesis. Therefore, prevention and treatment effects are discussed together.

3.5.1 Preclinical Studies

The role of flaxseed and its components in colorectal cancer cells have been investigated *in vitro* and *in vivo* but the results are conflicting (Table 3.3). Habermann et al. (2009) showed in both highly transformed HT29 colorectal cancer

Table 3.3 Summary of studies investigating effect of flaxseed, flaxseed oil and ALA on colorectal cancer

Model	Treatments/measures	Results	References
In vitro studies			
LT97 (adenoma) and HT29 (carcinoma)	100 μM ALA 2–72 h treatment	↓ cell growth in both cell lines, LT97 to a greater extent	Habermann et al. (2009)
Colorectal cancer cell line HCT116	Cells treated with 10 μM ALA	↑ cell number by 30 %	Seti et al. (2009)
In vivo animal studies			
Sprague-Dawley male rats with AOM-induced tumors	BD, 5 or 10 % FS flour (FF; 1.9–3.8 % FO) or defatted FS meal (FM; 0.14–0.28 % FO)	↓ Aberrant crypts (AC) and aberrant crypt foci (ACF) in the descending colon in all groups *vs*. control	Serraino and Thompson (1992b)
	Diets fed starting in early promotion stage	↓ in AC and ACF in the ascending colon in the 10 % FF group *vs*. control	
		↓ cell proliferation in descending colon in 5 % FF group compared to control; ↓ in 5 % FF, 10 % FM and 10 % FF *vs*. 5 % FM	
Male Fischer rats with AOM induced tumors (initiation and promotion)	15 % corn oil *vs*. 15 % FSO diets Fed diets during initiation and promotion stages	↓ tumor incidence, size and number per rat in FSO group *vs*. corn oil	Dwivedi et al. (2005)
Male Fischer rats with AOM induced tumors (initiation and promotion)	15 % corn meal *vs*. 15 % FS meal diets Fed diets during initiation and promotion stages	↓ tumor incidence, size and number per rat in FS group *vs*. corn meal	Bommareddy et al. (2006)
Male Fischer rats with AOM induced carcinogenesis (initiation and promotion)	Control, 7 and 14 % soybean oil (SBO), 7 and 14 % FSO, 10 and 20 % FSM diets Fed diets during initiation and promotion stages	↓ ACF in 7 and 14 % FSO group and FSM groups compared to 7 and 14 % SBO groups ↓ ACF in 20 % FSM *vs*. 10 % FSM ↑ ACF in 14 % FSO *vs*. 7 % FSO	Williams et al. (2007)
Min mice	BD, 15 % FS diet (defatted FSM + FSO) and FSO diet Started at 5 weeks age	↓ # adenomas in FS compared to control; non-significant ↓ in FSO ↓ adenoma size in FS and FSO *vs*. control	Oikarinen et al. (2005)

(continued)

Table 3.3 (continued)

Model	Treatments/measures	Results	References
Min mice	BD, 15 % corn meal *vs.* 15 % FS meal diets and 15 % corn oil *vs.* 15 % FSO	↓ intestinal tumor multiplicity and size in FSM compared to corn meal and FSO compared to corn oil No effect on apoptosis	Bommareddy et al. (2009)
Clinical and epidemiological studies			
Normal and tumor tissue samples from nine colorectal cancer patients	Free fatty acids of cell membranes	↓ ALA % content in tumor tissue *vs.* control	Szachowicz et al. (2007)
Case-control; 3,166 controls, 1,597 adenoma polyp cases, 544 hyperplastic polyp cases	Dietary PUFA intake measured by FFQ	↑ polyp occurrence with ALA intake in men, OR = 1.51 (1.03, 2.21), *p* trend = 0.03	Murff et al. (2012)
Case control; 74 cases, 221 controls	Erythrocyte fatty acid content	No association with colorectal cancer risk and erythrocyte ALA content, OR = 1.18 (0.63–2.21), *p* trend = 0.51	Kuriki et al. (2006)
Prospective cohort; 99,080 subjects	PUFA intake and colorectal cancer risk	↑ trend in colorectal cancer risk in women with ↑ ALA intake (*p* trend = 0.13)	Daniel et al. (2009)
RCT; 523 patients with colorectal adenomas	2 g/day calcium, 3.5 g/day fibre, or placebo Questionnaires for dietary fat intake	No association between colorectal adenoma reoccurrence and ALA intake, OR = 0.87 (0.52–1.46), *p* trend = 0.43	Methy et al. (2008)
RCT; 2,079 (372 fully completed) participants with colon polyps	Low fat, high fiber, diet or control Four day food records, FFQ and 24 h recalls	No association with colorectal adenoma recurrence and ALA intake OR = 0.92 (0.48–1.78), *p* trend = 0.87	Cantwell et al. (2005)

AC aberrant crypt, *ACF* aberrant crypt foci, *ALA* α-linolenic acid, *AOM* azoxymethane, *BD* basal diet, *FF* flaxseed flour, *FFQ* food frequency questionnaire, *FM* defatted flaxseed meal, *FS* flaxseed, *FSO* flaxseed oil, *OR* odds ratio, *PUFA* polyunsaturated fatty acid, *RCT* randomized controlled trial, *SBO* soybean oil

cells and preneoplastic LT97 adenoma cells that ALA was taken up by the cells and growth was inhibited when treated with 100 μM ALA for 72 h. Contrary to these findings, Seti et al. (2009) observed that 10 μM ALA increased HCT1116 colorectal cancer cell growth. Limitations in the current *in vitro* studies include variation in cell lines and doses used, and different stages of cancer progression.

To date rodent studies have focused specifically on the role of flaxseed and its oil in the prevention rather than treatment of colorectal cancer (Table 3.3). Serraino and Thompson (1992b) were the first to investigate the role of dietary flaxseed in the prevention of colon carcinogenesis. Using an azoxymethane (AOM)-induced rat model, they showed that feeding diets rich in flaxseed, either 5 or 10 % FF (ground whole flaxseed) or 5 or 10 % FM (defatted flaxseed), for 4 weeks during the promotion stage of colon carcinogenesis significantly reduced the incidence of aberrant crypts (AC) and aberrant crypt foci (ACF) in the descending colon compared to rats fed a control diet. Only the 10 % FF diet decreased AC and ACF incidence in the ascending colon compared to control indicating a greater effect of oil-containing flaxseed than defatted flaxseed. In a similarly designed study, Jenab and Thompson (1996) showed that feeding AOM-induced rats diets containing 2.5 or 5 % FF (ground whole flaxseed) or 2.5 or 10 % FM (defatted flaxseed) for approximately 14 weeks in the promotion stage reduced the number of AC per ACF in the distal colon. In contrast to the Serraino and Thompson study, only the 2.5 % defatted flaxseed diet reduced the number of ACF in the proximal colon compared to control. Other studies have also shown that both ground whole flaxseed (Bommareddy et al. 2006) and flaxseed oil (Dwivedi et al. 2005) fed to rats 1 week before and for 35 weeks following AOM administration significantly reduced tumor incidence, multiplicity and size when compared to corn meal and corn oil respectively. Similarly, ground whole flaxseed and flaxseed oil fed for 4 weeks before and for 10 weeks following AOM administration reduced the number of ACF in the proximal, distal and total colon compared to the soybean oil control (Williams et al. 2007). Together these data suggest that flaxseed has components such as the oil capable of inhibiting the initiation and promotion stages of colon carcinogenesis in the rat model.

The Min mouse model which has a mutation in the Adenomatous polyposis coli (*APC*) gene spontaneously develops tumors and has been useful in further understanding the role of flaxseed and its oil in colon carcinogenesis. Compared to BD, feeding the BD enriched with a flaxseed mixture (2.6 % defatted flaxseed + 4.7 % flaxseed oil) or flaxseed oil (4.7 %) to Min mice for 10 weeks resulted in a significant decrease in adenoma size and number, although the reduction in adenoma multiplicity was only significant in the flaxseed mixture group (Oikarinen et al. 2005). Similarly, it was shown that 15 % ground whole flaxseed and 15 % flaxseed oil diets fed for 12 weeks to Min mice suppressed intestinal multiplicity and size compared to feeding 15 % corn meal and 15 % corn oil diets respectively (Bommareddy et al. 2009). These results further support the role of dietary flaxseed and flaxseed oil in the prevention of colon cancer.

3.5.2 Clinical and Epidemiological Studies

Data collected from both clinical and epidemiological studies on flaxseed, flaxseed oil and ALA and colorectal cancer have raised concern as several studies show no effect or protection, while others show an increase in colorectal cancer risk (Table 3.3). One prospective cohort study showed that ALA intake measured by a

FFQ was associated with an increase in colorectal cancer incidence in women only, however flaxseed oil supplements were omitted from the FFQ and ALA intake was primarily from non-plant sources (Daniel et al. 2009). Similarly in a case-control study, ALA intake measured *via* FFQ showed that increasing ALA intake was associated with an increase in hyperplastic polyps in men (Murff et al. 2012).

Contrary to the above studies, ALA content in cell membranes of normal colorectal tissue has been shown to be higher than that of tumor tissue (Szachowicz-Petelska et al. 2007). In a systematic review of fatty acids and colorectal cancer, two case control studies showed that ALA content was lower in cancer patients compared to controls indicating a protective effect, however one study did find an increase in ALA intake in subjects at high risk of colorectal cancer (Nkondjock et al. 2003b). Other case control and cohort studies all found no significant association of ALA and colorectal cancer incidence (Nkondjock et al. 2003b). Clinical trials would be useful to help resolve the controversy surrounding flaxseed and its components in colorectal cancer; however, this area of research is currently lacking. Two randomized control trials which focused on the effect of either a calcium or fiber supplement, or a low fat, high fiber, fruit and vegetable diet on colorectal adenoma recurrence indirectly found that ALA intake did not have a significant association with colorectal cancer incidence as measured by questionnaires and food records (Cantwell et al. 2005; Methy et al. 2008). Limitations in the current epidemiological and clinical studies which may explain the inconsistent results are the use of unreliable FFQs for intake measurements, variation in biomarkers measured, not stating specific colon/rectal subsite location, and variation in the food source of ALA.

3.6 Studies of Flaxseed and Flaxseed Oil on Prostate Cancer

Similar to colorectal cancer, fewer studies have investigated the role of flaxseed and its components on prostate carcinogenesis compared to breast/mammary carcinogenesis, therefore, this section will discuss prevention and treatment studies together.

3.6.1 Preclinical Studies

The few *in vitro* studies that have investigated the effect of ALA on prostate cancer cells have produced contradictory results (Table 3.4). Two studies using DU 145 human prostate tumor cells found that physiological levels of ALA decreased cell proliferation and increased the number of dead cells (du Toit et al. 1996; Motaung et al. 1999). However, one study using human metastatic prostate cell lines PC-3, LNCaP and TSU found that ALA actually promoted cell growth (Pandalai et al. 1996). Future studies should continue to investigate the role of ALA on prostate cell

Table 3.4 Summary of studies investigating effect of flaxseed, flaxseed oil and ALA on prostate cancer

Model	Treatments/measures	Results	References
In vitro studies			
Metastatic PC-3, LNCaP, TSU cells	0.003–0.359 μM	↑ cell growth in all cell lines	Pandalai et al. (1996)
DU-145 prostate tumor cells	4, 40, 200 μM ALA Six days	↓ cell growth with 40 and 200 μM ALA for 6 days	Toit et al. (1996)
DU-145 prostate tumor cells	4, 20, 40 μM ALA	↑ cell death with 20 μM and 40 μM ALA	Motaung et al. (1999)
In vivo animal studies			
TRAMP transgenic model	BD, 5 % FS Started at 5–6 weeks age Followed for 20 or 30 weeks	↓ urogenital/tumor weight, incidence of aggressive tumors at 30 weeks ↓ cell proliferation and ↑ apoptosis at both 20 and 30 weeks	Lin et al. (2002)
Male athymic nude mice with DU145 human prostate cancer xenografts	18 % corn oil/5 % FSO; 18 % FSO/5 % corn oil; 18 % fish oil/5 % corn oil Fed diets starting before implantation	No difference in tumor growth between the 18 % CO/5 % FSO; 18 % FSO/5 % CO diets ↓ tumor growth in the fish oil group compared to both other groups	Connolly et al. (1997)
Wistar rats (no carcinogen administration)	7 % soybean oil (SBO), 7 % SBO/FSO (1:1), 7 % FSO, 7 % SBO/pork fat (1:1), 7 % pork fat Fed for 10 weeks	↓ relative prostate weight in 7 % FSO group *vs.* SBO control and SBO/FSO ↑ cell proliferation in prostate tissue in lard group *vs.* SBO and FSO groups	Escobar et al. (2009)
Clinical and epidemiological studies			
Nested case control; 962 prostate cancer patients, 1,061 controls	Fatty acid composition of plasma phospholipids	No association with prostate cancer risk and % ALA; ↑ high grade cancer risk with ↑ % ALA (p trend 0.014)	Crowe et al. (2008)
Systematic review; eight case control, eight prospective	ALA intake or blood concentrations	↑ prostate cancer risk with ALA blood concentrations, RR = 1.20 (1.01–1.43), no association with dietary intake	Simon et al. (2009)
Meta analysis; five prospective studies	FFQ for ALA intake	No association with prostate cancer risk and ALA intake, pooled RR = 0.97 (0.86–1.10) If consumed >1.5 g/day ALA ↓ risk, RR = 0.95 (0.91–0.99)	Carayol et al. (2010)

(continued)

Table 3.4 (continued)

Model	Treatments/measures	Results	References
Meta analysis; nine case control and cohort	ALA intake and blood level	↑ prostate cancer risk with ALA intake and blood levels, RR = 1.70 (1.12–2.58)	Brouwer et al. (2004)
Pilot Study, 15 healthy men	Low fat, FS (30 g/day) diet for 6 months	↓ cell proliferation with low fat FS diet (0.022 ± 0.027 baseline to 0.007 ± 0.014 at 6 months, $p = 0.0168$)	Demark-Wahnefried et al. (2004)
Pilot clinical study, 25 prostate cancer patients	Low fat, FS (30 g/day) diet for 34 days	↓ proliferation in treatment group (5.0 ± 4.9 treatment, 7.4 ± 7.8 for control, $p = 0.05$) ↑ apoptosis in treatment group $p = 0.01$	Demark-Wahnefried et al. (2001)
RCT; 161 prostate cancer patients	Control, FS diet (30 g/day), low fat (<20 % energy), combination ; 30 days average	↓ proliferation in FS group ($p < 0.002$) No effect on apoptosis	Demark-Wahnefried et al. (2008)

ALA α-linolenic acid, *FS* flaxseed, *FSO* flaxseed oil, *RCT* randomized controlled trial, *RR* relative risk, *SBO* soybean oil, *TRAMP* transgenic adenocarcinoma of mouse prostate

growth, and take into account differences between cell lines, doses used and other components in the media environment that may alter ALA effects.

Animal models have shown varying effects of flaxseed and flaxseed oil rich diets on the growth of prostate tissue and tumor growth (Table 3.4). Current studies have focused on prostate cancer prevention. Healthy male Wistar rats fed a 7 % flaxseed oil diet had lower relative prostate weight compared to a 7 % soybean oil diet and lower relative prostate weight and prostate cell proliferation compared to a 7 % rendered pork fat diet rich in saturated fat (Escobar et al. 2009). Using the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, a 5 % flaxseed diet was shown to reduce the urogenital/tumor weight, number of aggressive tumors and prostate tissue cell proliferation and increase apoptosis after 30 weeks (Lin et al. 2002). On the other hand, an early study compared the effects of diets rich in corn oil (18 % corn oil/5 % flaxseed oil), flaxseed oil (18 % flaxseed oil/5 % corn oil) and fish oil (18 % menhaden oil/5 % corn oil). When fed 1 week before injection of DU145 human prostate cancer cells in athymic mice the diet with greater flaxseed oil did not affect tumor growth while the fish oil rich diet reduced tumor growth (Connolly et al. 1997). The major differences in design of these animal studies make it difficult to compare them, however, based on results in the healthy rats and transgenic mice, there is some indication that flaxseed and flaxseed oil may prevent the development of prostate cancer.

3.6.2 Clinical and Epidemiological Studies

Several reviews and meta-analyses have been conducted on the role of ALA in prostate cancer development with conflicting results (Brouwer et al. 2004; Simon et al. 2009; Carayol et al. 2010) (Table 3.4). Brouwer et al. (2004) found that increasing ALA dietary intake and/or blood level significantly increased prostate cancer risk in five case control and four cohort studies. Simon et al. (2009) pooled eight case control and nine prospective studies and found a weak association between ALA (dietary intake or concentration in tissues) and increased prostate cancer risk. Carayol et al. (2010) looked specifically at five prospective cohort studies and the pooled relative risk showed no significant association between ALA intake and prostate cancer; however, it was found that those who consumed more than 1.5 g/day of ALA were actually at a decreased risk of prostate cancer compared to those below 1.5 g/day. The European Prospective Investigation into Cancer and Nutrition (EPIC) also investigated the role of plasma phospholipid fatty acid composition on prostate cancer in a nested case-control study and found ALA composition of the plasma phospholipids was not significantly associated with prostate cancer risk, with the exception of high-grade prostate cancer in which ALA increased risk (Crowe et al. 2008). Limitations of these studies overall include the use of unreliable FFQs to measure ALA intake, the food source of ALA and significant heterogeneity across some studies and highlight the need for controlled clinical trials to resolve the controversy.

In a clinical pilot intervention study, 15 men scheduled for a repeat prostate biopsy adopted a low fat (<20 % of energy), flaxseed supplemented (30 g/day) diet for 6 months prior to second biopsy and showed that cell proliferation of the benign prostate epithelium slightly decreased with the low fat, flaxseed diet from baseline to 6 months, indicating flaxseed may prevent prostate cancer (Demark-Wahnefried et al. 2004). Two clinical trials have assessed the ability of a flaxseed supplemented diet prior to prostatectomy to act as a potential treatment through measurement of cell proliferation and apoptosis of prostate cancer cells from the excised prostate tumors of prostate cancer patients (Demark-Wahnefried et al. 2001, 2008). The 25 patient pilot study found that a low fat (<20 % of energy), flaxseed supplemented (30 g/day) diet for ~30 days prior to prostatectomy decreased cell proliferation and increased apoptosis compared to matched historic cases, indicating a decrease in cancer cell growth. The follow-up randomized control study in 161 prostate cancer patients found that a 30 g/day flaxseed diet for 30 days significantly decreased cell proliferation compared to control ($p < 0.002$), although no significant difference was seen on apoptosis, suggesting that flaxseed may provide a benefit in reducing cancer cell growth in prostate cancer patients (Demark-Wahnefried et al. 2008). Further studies are required to provide a better understanding of the effect of flaxseed, flaxseed oil and ALA on prostate cancer and potential mechanism of effect.

3.7 Studies of Flaxseed and Flaxseed Oil in Other Cancers

The effect of flaxseed and its components have also been determined in other cancers but the studies are limited and results are inconsistent. A study using laying hens as a model for spontaneous ovarian surface epithelial cancer found that a 10 % flaxseed diet fed for 1 year decreased late stage tumors and increased survival compared to the control diet, suggesting protective effect against ovarian cancer (Ansenberger et al. 2010). In pancreatic cancer cell lines (MIS PaCa-2, PANC-1 and CFPAC), ALA in doses of 10–20 μM significantly decreased cell number across the three cell lines indicating a beneficial effect (Falconer et al. 1994). In contrast, in Syrian golden hamster models of BOP-induced pancreatic ductular carcinoma, liver metastases occurred when 2.5–10 % ALA was incorporated into the diet (Wenger et al. 1999, 2000). However, the diets in these studies varied greatly in fat content (3 % fat control diets *vs*. 25 % fat treatment diets), carbohydrate to fat ratio, and protein and fibre contents so conclusions made regarding increased liver metastases may not necessarily be due to dietary ALA. Two other studies also looked at the effect of flaxseed and flaxseed oil on metastasis to the liver and lungs in animal models. In C57B1/6 mice with murine melanoma cells injections, a 2.5–10 % flaxseed diet 2 weeks pre and post melanoma cell injection dose dependently decreased the number, area, and volume of secondary lung tumors (Yan et al. 1998). In the same C57B1/6 mouse model using H59 lung carcinoma cells; however, an 8 % flaxseed oil diet 4 weeks pre injection and roughly 5 weeks post injection increased metastasis to the liver compared to a control no fat diet, a saturated fat diet, and an n-6 PUFA diet (Coulombe et al. 1997). These limited and contradictory studies highlight the need for further work in the area of flaxseed supplementation for a variety of cancer types.

3.8 Proposed Mechanisms of Anticancer Effect

The anticancer effect of flaxseed oil is thought to be due to ALA which is the predominant fatty acid. The mechanisms by which ALA modulates carcinogenesis are not yet understood although several have been proposed (Fig. 3.3). ALA's effect was thought to be due to both direct effects and indirect effects through conversion to EPA and DHA (Fig. 3.1). However, the conversion of ALA to EPA and DHA is quite low although humans have functional enzymes for the metabolic pathway for the conversion (Cunnane 2003; Brenna et al. 2009). Nevertheless, flaxseed, flaxseed oil and ALA have all been shown to have anticancer properties and hypothesized mechanisms of effect include: (1) incorporation into the cancer cell membrane thus (1a) increasing the synthesis of n-3-derived eicosanoids and (1b) disrupting the localization, expression and signalling of growth factor receptors; (2) alteration of the regulation of transcription; (3) increased lipid peroxidation; and (4) other emerging potential mechanisms (Fig. 3.3).