Chapter 5
Soy Foods: Towards the Development of Novel Therapeutics for Breast Cancer

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Abstract The increasing cognizance that diet (and lifestyle) can modify breast cancer risk and progression has motivated many breast cancer patients to take increasing personal control of the direction of their therapies after diagnosis and surgery. While this has certain advantages, including higher compliance to prescribed drugs and improvements in emotional and mental well-being, it predicates the need for increased understanding of the benefits of particular diets and dietary regimen to the treatment programs and for improved translation of data obtained from studies with animal models into clinical settings. Epidemiological studies have linked high consumption of soy-rich foods to the lower incidence of breast

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cancer in Asia relative to that in Western countries. The potential of soy-rich foods as breast cancer protective when dietary exposure occurs early in life, has resulted in driving the use of soy and its associated bioactive components, specifically the isoflavone genistein, as chemopreventive agents or as adjuvants to conventional drug therapies. Bioactive components in soy foods may affect hormone and non-hormone-mediated mechanisms. However, their overall biological outcomes remain not well-understood and at times, contradictory, due to distinct physiological contexts and doses of exposure, multiple targets, and inconsistent measures of relevant endpoints. Here we provide an argument in support of the potential use of soy foods for breast cancer patients based on the review of the current literature as well as raise caveats that must be addressed for its successful application as standard-of-care treatment.

5.1 Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer deaths among women in the United States (Siegel et al. 2012). Worldwide, more than 450,000 new cases of breast cancer are diagnosed annually, and the numbers of women who succumbed to the disease have tripled within the past three decades. Nevertheless, there is a disparity in the global distribution of breast cancer, a consequence in part of environmental rather than genetic differences among the general population (Hortobagyi et al. 2005). Diet and lifestyle constitute modifiable determinants of breast cancer risk (Blackburn et al. 2003; Brennan et al. 2010; Patterson et al. 2010). The strongest support for the notion that breast cancer susceptibility can be influenced by nutrition and lifestyle has come from epidemiological and case–control studies demonstrating a two- to eight-fold lower occurrence of breast cancer in Asian women, whose early intake of soy foods is 10–20 times higher than their Western counterparts (Shu et al. 2001; Hilakivi-Clarke et al. 2010). Based on the latter and the emerging evidence for diet-mediated regulation of mammary epithelial differentiation, proliferation, and apoptosis, either directly or circuitously (Su et al. 2011), the prospect that soy foods and associated bioactive components may constitute novel therapeutics for breast cancer is a definite possibility. Indeed, the current interest in soybeans and their phytoestrogen components have triggered a number of limited clinical trials (http://www.clinicaltrials.gov) to evaluate the efficacy of these compounds as treatment modalities in women afflicted with the disease.

Natural agents found in foods may, theoretically, confer benefit for breast cancer control in two ways, which are not necessarily exclusive: one, by acting as chemopreventive agents, to inhibit, delay and reverse the development and progression of the disease, and second, as a drug to sensitize tumor cells to conventional therapies (chemotherapy, radiation treatment) and impede further progression, recurrence, and metastasis. The over-arching goal of these interventions is to decrease breast cancer risk, increase patient survival, and improve quality of life after

Table 5.1 Pro-survival and pro-death pathways influenced by exposure to bioactive components in soy foods

Signaling pathway	Pro-survival mediators	Pro-death mediators
Wnt/β-catenin	β-catenin	BAX
	Bcl2	E-cadherin
	C-myc	p21
	Cyclin D1	
PI3K/PKB(AKT)	mTOR	PTEN
NF-κB	Interleukins	p65
	TNFα	
p53	Survivin	p53
IGF-1	PKB (AKT)	IGFBP3
	mTOR	
	PI3K	
	MAPK	
	JNK	
Estrogen receptor mediated	ERα	ERβ
BRCA mediated		BRCA1, BRCA2

BRCA1 breast cancer type 1 susceptibility protein, *ER* estrogen receptor, *IGF-1* insulin-like growth factor 1, *IGFBP* insulin-like growth factor binding protein, *JNK* jun kinase, *MAPK* mitogen activated protein kinase, *mTOR* mammalian target of rapamycin, *NF-κB* nuclear factor-kappa B, *PI3K* phosphoinositide 3-kinase, *PKB* protein kinase B (also known as AKT), *PTEN* phosphatase and tensin homologue, *TNFα* tumor necrosis factor alpha

breast cancer. How cells integrate the cellular signals imposed by dietary factors and respond accordingly under distinct physiological contexts remains unclear. Because the major consequences of these regulatory signals may differ between normal and neoplastic breast cells, it is imperative that clinicians and healthcare professionals with the intent of harnessing the bioactivities of dietary constituents for therapeutic interventions understand the central molecular players that orchestrate response to pro-death and pro-survival signals (Table 5.1) that are induced and repressed, respectively by bioactive soy components.

In this chapter, we discuss the preclinical and clinical studies that provide support for (or against) the use of soy foods as endocrine or local paracrine interventions that may dictate the fate of breast cancer cells to arrest growth and die. We also briefly present here, the biological mechanisms currently considered to mediate dietary effects on distinct mammary compartments.

5.2 Mammary Gland Biology and Mechanisms of Dietary Protection

The mammary gland, structurally and developmentally, is one of the most complex tissues in mammals. It is comprised of myoepithelial and luminal epithelial cells embedded in a complex stromal matrix (so called mammary fat pad), composed

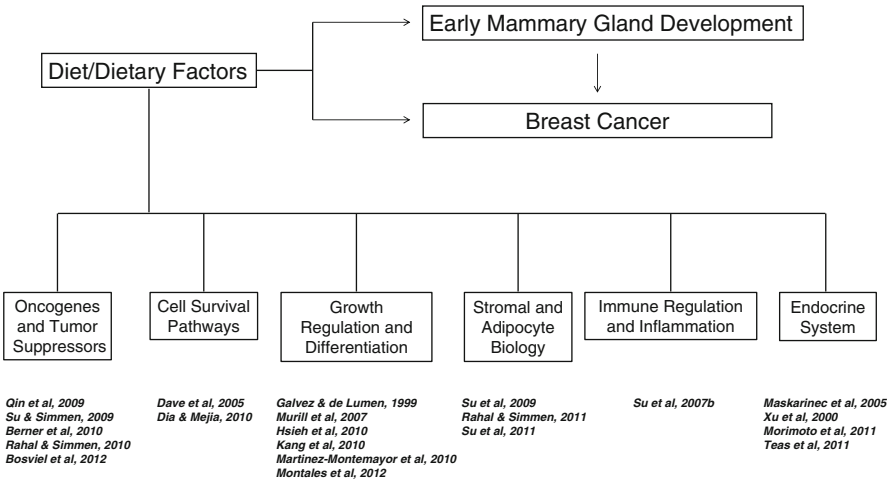

Fig. 5.1 Summary of proposed biological events regulated by soy and associated bioactive components in mammary epithelial and stromal compartments for breast cancer protection. Dietary factors influence numerous processes during distinct stages of early mammary gland development that are subverted due to genetic mutations and epigenetic modifications during breast cancer initiation and progression. Representative publications providing scientific support to signaling pathways that are influenced by diet are cited here and listed under References

predominantly of fibroblasts, adipocytes and macrophages. While the development of the mammary gland differs temporally to some extent in human females and rodents (Hennighausen and Robinson 2001), it is widely acknowledged that the dynamic growth, organization and structuring of the epithelial compartments in both species occur at puberty with the onset of ovarian estrogen synthesis. Nevertheless, the key (and paradigm-shifting) findings that a mammary epithelial hierarchy exists (Shackleton et al. 2006); that the epithelial sub-population 'sitting at the top' of the mammary epithelial hierarchy can serve as initial targets of oncogenic agents (Visvader 2009), and that fetal mammary glands (in mice) contain a higher population of mammary stem cells than in adult mammary tissues (Spike et al. 2012) implicate mammary epithelial cells from which tumors arise and neighboring stromal cells to exhibit remarkable plasticity beginning from the earliest stages of mammary development. Thus, the study of events leading to breast cancer initiation and progression and of how diets can influence breast cancer risk is tightly coupled to the understanding of dietary effects on early mammary gland development. The plethora of local- and endocrine-derived factors that regulate the transcriptional programs in mammary epithelial cells and of the neighboring stromal cells is beyond the scope of this chapter. However, the signaling pathways regulated by dietary factors that allow for normal functions and development of the mammary gland are most likely the same as those that become deregulated leading to breast cancer (Fig. 5.1).

5.3 Soy Food Intake and Breast Cancer Prevention

Evidence suggests that soy food intake during childhood and adolescence is breast cancer protective in later life (Shu et al. 2001; Wu et al. 2008a, b; Korde et al. 2009; Lee et al. 2009). Of importance, the protective effects of early soy intake during childhood were stronger and more consistent than intake at any other life stage (Korde et al. 2009) and found to be equally effective for both pre- and post-menopausal breast cancers (Shu et al. 2001; Lee et al. 2009). These observations are aligned with the concept of developmental plasticity originally proposed by Prof. Barker (2007) based on epidemiological data, suggesting critical periods during very early development that are vulnerable to environmental factors, including diet. Studies on rodent models of breast cancer have provided support for the human observations (Lamartiniere 2002; Hilakivi-Clarke and De Assis 2006; Murill et al. 2007; Su et al. 2007a). Exposure of developing rat mammary glands to soy bioactive components, primarily the soy isoflavone genistein (GEN), reduced the number of terminal end buds and increased the number of differentiated lobules in young adult rat mammary glands, indicative of an early increase in mammary tissue differentiation, a well-accepted mechanism for protection against mammary tumorigenesis. Our own group has shown using chemically-induced (*N*-methyl-nitrosourea) mammary tumor formation in rats, that lifetime dietary exposure (i.e. beginning *in utero* through adulthood) to soy protein isolate (SPI) containing GEN reduced tumor incidence and increased tumor latency (Simmen et al. 2005); this was accompanied by an early increase in tumor suppressor phosphatase and tensin homologue (PTEN) deleted on chromosome ten expression (Dave et al. 2005) and a concomitant decrease in the tumor oncogene β-catenin signaling (Su et al. 2007b). PTEN, next to p53 is the most common tumor suppressor to be lost or inactivated in human cancers, including breast cancer (Li et al. 2002) and functions to antagonize the phosphatidylinositol-3-kinase (PI3K), thus, preventing the activation of the pro-survival protein kinase B/Akt downstream pathway (Stambolic et al. 1998). A consequence of PTEN loss is apoptosis-resistance and decreased differentiation, both hallmarks of cancer cells (Hanahan and Weinberg 2011). On the other hand, defective pathways in Wnt signaling lead to the stabilization of nuclear β-catenin pools, resulting in uncontrolled proliferation, and are associated with >50 % of breast carcinomas (Lin et al. 2000). Studies from our group using mammary epithelial cell lines *in vitro* confirmed the GEN effects noted in the animal studies and provided mechanistic insights for GEN-mediated induction of PTEN expression and activity (Dave et al. 2005; Rahal and Simmen 2010) and inhibition of Wnt-signaling (Su and Simmen 2009). These mechanisms are summarized in Fig. 5.2.

How may early exposure to soy foods and soy isoflavones promote mammary differentiation, leading to breast cancer protection in women at adulthood? Qin and colleagues (2009) in a study of healthy premenopausal women implicated the ability of soy isoflavones to increase the methylation of several cancer-related

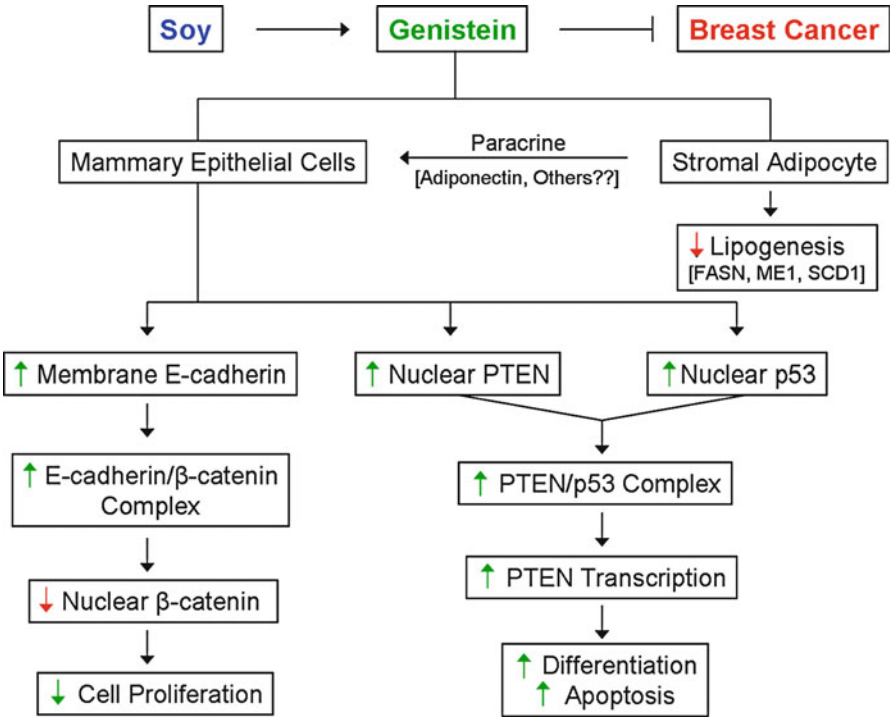

Fig. 5.2 Schematic representation of experimentally-defined mechanisms underlying the protective effects of the soy isoflavone genistein against breast cancer. Human mammary epithelial cells and rat mammary glands were used in these studies, as described in detail in Su and Simmen (2009), Su et al. (2009), and Rahal and Simmen (2010).

genes as potential mechanisms for mammary tumor protection. These include: the cyclin-dependent kinase inhibitor 2A (*p16*), tumor suppressor retinoic acid receptor B2 (*RARB2*), estrogen receptor-α (*ER-α*), and cyclin D2 (*CCND2*), a tumor oncogene by virtue of its inhibition of tumor suppressor retinoblastoma (Rb) protein function. GEN's activity to alter promoter hypermethylation in human breast tissues provides *in vivo* support for epigenetic underpinnings to its anti-breast cancer risk activity. In this regard, transcriptional networks in the mammary gland are widely acknowledged to be regulated by chromatin architecture and promoter DNA modifications (Rijnkels et al. 2010). Soy phytoestrogens GEN and daidzein have been shown to reverse DNA hypermethylation of tumor suppressors *BRCA1* and *BRCA2* in breast cancer cell lines (Bosviel et al. 2012) and GEN, similar to the natural polyphenolic compound resveratrol, increased promoter methylation of *ER-α*, coincident with this gene's increased expression (Berner et al. 2010). Similarly, methylation patterns in mice (Day et al. 2002) and cynomolgus monkeys (Howard et al. 2011) were altered by dietary GEN or soy consumption. GEN, daidzein and equol have also been shown to modify histone marks (by acetylation and demethylation) in target genes, including *BRCA1* to

modify their transcription (Dagdemir et al. 2013). GEN-mediated enhancement of mammary PTEN expression during early mammary gland development coincident with mammary tumor protection in rats (Dave et al. 2005), is likely related to GEN's role in altering promoter methylation, since increased methylation of *PTEN* gene promoter was associated with increased breast cancer invasion and metastasis (Liu and Yang 2011). GEN exposure does not appear to affect global DNA methylation (Vanhees et al. 2011), however, indicating that its selective epigenetic impact in mammary chemoprevention might involve the reversal of adverse epigenetic marks in a minority of mammary epithelial subpopulations. This small subset of cells, designated as mammary stem cells, give rise to functional mammary glands and when deregulated, can initiate mammary tumors (Stingl et al. 2006; Visvader 2009). The elucidation of the effects of bioactive compounds associated with soy foods on this epithelial subpopulation is a major focus of ongoing studies in our group (Montales et al. 2012). In this regard, work from our laboratory suggest that post-wean intake of soy protein isolate (as sole protein source) or of GEN added to control (casein) diet at concentrations approximating those found in soy foods, reduced mammary tumor incidence, relative to casein in a mouse model of human breast cancer. This was accomplished in part, by reducing the mammary stem cell-enriched population and in particular, the cancer stem cell population in hyperplastic tissues of mice overexpressing the Wnt oncogene (e.g. MMTV-Wnt1 transgenic mice) only in mammary tissues.

5.4 Soy Food Intake and Breast Cancer Survival

A lingering question related to soy food intake is whether breast cancer survivors who are receiving adjuvant endocrine therapy should include or exclude soy foods as part of their normal diets. This question stems from the lack of understanding of whether and how the weak estrogenic properties of isoflavones might interfere with conventional therapies (e.g. tamoxifen, anastrozole), leading to the promotion of breast cancer recurrence and mortality. In a 2004 article, Nair presented several case reports of cancer survivors who showed significant improvements in their conditions after dietary supplementation with a fermented soy product Haelan951 either as sole treatment or as adjuvant nutrition. While the reported cancer cases included only one breast cancer patient with infiltrating ductal carcinoma, the data provided support for the benefits associated with the dietary supplementation of fermented soy. The collective review of the more recent literatures (2003 and later) with median follow-up of 3 years or greater, has largely demonstrated the significant reductions in breast cancer risk or recurrence with high dietary intake of soy isoflavones through regular consumption of soy foods (Suzuki et al. 2008; Guha et al. 2009; Shu et al. 2009; Caan et al. 2011; Dong and Qin 2011; Kang et al. 2012; Woo et al. 2012). The specific dietary interventions and findings from a number of such studies with breast cancer patients are summarized in Table 5.2. Differences in outcomes are likely due to differing intervention designs, duration of dietary intake,

Table 5.2 Representative studies on the effects of soy and isoflavone intake on breast cancer incidence, breast cancer recurrence, and mortality in women with breast cancer

	Demographic data					
Study	Status	Total women in study	Length of follow-up	Intake	Outcome (incidence, recurrence, mortality)	Findings
Yamamoto et al. (2003)	With breast cancer	21,852	NR	Iso	Incidence	IA (S)
Nishio et al. (2007)	With breast cancer	30,454	7.6 y	Iso	Incidence	NA
Wu et al. (2008a, b)	With breast cancer	35,303	NR	Iso	Incidence	IA (S)
Guha et al. (2009)	Breast cancer survivor	1,954	6.3 y	Iso	Recurrence	IA (S)
Shu et al. (2009)	Breast cancer survivor	5,042	3.9 y	Soy, Iso	Recurrence, mortality	IA (S)
Kang et al. (2010)	Breast cancer survivor on adjuvant therapy	542	5.1 y	Iso	Recurrence, mortality	IA with recurrence (S); NA with mortality
Caan et al. (2011)	Early stage breast cancer	3,088	7.3 y	Iso	Recurrence, mortality	IA (S)
Kang et al. (2012)	With breast cancer	256	5 y	Soy	Mortality	IA (S)
Nechuta et al. (2012)	Breast cancer survivor	9,514	7.4 y	Iso	Recurrence, mortality	IA (NS)

IA inverse association, *Iso* isoflavone, *NA* no association, *NR* not reported, *NS* non-significant, *S* significant, *y* year

menopausal status, and race (White or Asian). Nevertheless, it is important to note that for these reports, none found increased deaths or breast cancer recurrence with the interventions, suggesting the relative safety of soy food intake for breast cancer patients.

An example of a study providing a definitive message on the positive effect of regular soy food consumption is the report by Shu et al. (2009). Upon adjustments for known clinical predictors and other lifestyle factors, the authors found that intake of soy foods either as soy protein or soy isoflavone was inversely associated with mortality and recurrence. Importantly, the inverse association was found irrespective of estrogen receptor status, and use or non-use of tamoxifen. In other studies however, the benefits of soy isoflavones for decreasing risk of breast cancer recurrence have not proved clear-cut and appear to be highly dependent on physiological context, likely reflecting the complex spectrum of bioactivities of soy components. Guha et al. (2009) reported that the trends for reduction of breast

cancer recurrence among postmenopausal, tamoxifen users were positively associated with increasing intake of daidzein and glycetin, while Kang et al. (2010) found effects of soy isoflavones only among postmenopausal but not premenopausal patients. The improved efficacy of tamoxifen in combination with isoflavone daidzein, relative to tamoxifen alone in reducing mammary tumor formation was previously shown in rat models to be associated with decreased oxidative damage in mammary glands (Constantinous et al. 2005). On the other hand, low-dose GEN abrogated the inhibitory effect of tamoxifen on growth of MCF-7 mammary tumors explanted in ovariectomized athymic nude mice (Du et al. 2012). The study of Suzuki et al. (2008) highlighted receptor status among patients as a modifiable parameter for efficacy of soy dietary intake on reducing breast cancer recurrence. The authors found that reduced risk of breast cancer recurrence was observed only in patients with ER+/PR+/HER2− tumors. The latter is supported by the recent report that high intake of soy isoflavones increased breast cancer recurrence in HER2+ breast cancer patients (Woo et al. 2012). A most restrictive criterion for the benefits of soy and isoflavones in breast cancer came from the study by Dong and Qin (2011). Here, the inverse association between soy isoflavone intake and breast cancer recurrence was only observed in Asian but not in Western populations, suggesting that overall lifestyle differences, of which higher soy consumption is only one parameter, contribute to relative risks.

Given that breast cancer is generally a disease of old age and affects largely postmenopausal women, the finding that menopausal status is an important factor for the therapeutic value of soy food intake implicates endocrine effects of soy foods and isoflavones. The effects of soy consumption on endogenous estrogen metabolism have been reported (Xu et al. 2000; Morimoto et al. 2011). Interestingly, soy isoflavones altered estrogen metabolism in both pre- and post-menopausal women, suggesting that the response of mammary epithelial cells to changes in estrogen levels rather than estrogen levels itself may account for the differential effects. However, serum concentrations of other hormones are also altered by soy intake; these include serum insulin-like growth factor-1 (IGF-1) which is increased in pre- (Gann et al. 2005; Maskarinec et al. 2005) and post-menopausal (Teas et al. 2011) women, and follicle-stimulating hormone and luteinizing hormone which are similarly decreased in premenopausal women, in the absence of effects on menstrual cycle length (Duncan et al. 1999). These results are counter-intuitive and difficult to reconcile as potential mechanisms underlying reduction in breast cancer recurrence, since IGF-1 is pro-proliferative in epithelial cells. In this regard, intake of soy isoflavones has not been demonstrated to modify mammographic density, a strong marker of breast cancer risk, in postmenopausal women (Verheus et al. 2008; Maskarinec et al. 2009). By contrast, a modest increase in mammographic density was noted in premenopausal women (Hooper et al. 2010). Findings suggest that the exploration of specific cellular contexts of premenopausal *vs* postmenopausal breast epithelial cells that alter their steroid and growth factor responses is critical to our understanding of potential therapeutic strategies aimed at utilizing soy intake for improving breast cancer prognosis.

5.5 Bioactive Soy Components and Predictive Biomarkers

The growing repertoire of signaling pathways mediated by soy isoflavones, if validated, could serve as relatively straightforward predictive biomarkers for identifying patient populations potentially responsive to cellular actions of bioactive soy components. Histological analyses of mammary biopsies for proliferative (e.g. Ki-67) and apoptotic (caspase-3, Bcl2) markers, and tumor suppressors (e.g. PTEN) could provide indications of soy effects prior to and after treatments. Levels of estrogens in nipple aspirate fluids, rather than in serum, could be valuable as more direct indications of the impact of dietary soy and/or GEN exposure with tamoxifen therapy, on breast tissue due to changes in estrogen metabolism (Morimoto et al. 2011). In a randomized phase 2 trial involving 126 healthy, high risk adult Western women, fine needle aspiration was used for collection of mammary epithelial cells to evaluate the effects of mixed soy isoflavones or placebo prior to and after dietary supplementation for 6 months. Ki-67 labeling of the cells as well as the expression of genes related to proliferation, apoptosis and estrogenic effects were quantified. Despite the lack of demonstrable significant differences between control and treated groups for these measured parameters, suggesting lack of efficacy of soy isoflavones within the limited exposure time, the methodology highlights the value of these biomarkers to measure response rate in future study populations (Khan et al. 2012).

Given the increasing interest towards personalized therapy for breast and other types of cancer, other strategies are being developed to identify and validate biomarkers for chemotherapy and pathological complete response. Biomarkers identified and currently being assessed, in addition to the classical ER and Ki-67 expression, are the anti-apoptotic protein survivin and pro-proliferative phosphorylated ERK (by immunohistochemistry) in breast tissues (Sanchez-Rovira et al. 2012); apoptotic-related biomarkers (e.g. soluble cell death receptor sFAS, plasminogen activator inhibitor-1) in serum of breast cancer patients undergoing neoadjuvant therapy (Fersching et al. 2012); expression of epidermal growth factor receptor and topoisomerase II alpha (TOP2A) in circulating tumor cells (CTC) isolated using anti-CTC surface antigens (Nadal et al. 2012), and methylation signatures using a functional hypermethylome screen for breast tissue (Jeschke et al. 2012). In the latter study, methylation of tachykinin 1 precursor 1 (*TAC1*) and creatine kinase muscle (*CKM*) singly proved to be highly correlated with poor overall survival in breast cancer patients, and in combination, was strongly associated with poor overall survival independent of age. While studies to investigate soy effects on breast cancer patients using these putative prognostic markers maybe premature, the universal application of these promising technologies in future soy studies might streamline the variables in experimental design and outcome measurements that confound data interpretation in clinical studies carried out under different settings.

5.6 Challenges for Potential Therapeutic Exploitation of Soy Bioactive Components

While there is a paucity of information to directly link soy bioactive components and therapeutic outcome in breast cancer patients, there are sufficient information, as highlighted in recent meta-analysis of prospective and epidemiological studies cited in this chapter (Qin et al. 2006; Trock et al. 2006; Dong and Qin 2011) to support this possibility. However, there are several challenges associated with developing soy components for breast cancer therapy. The first challenge is to identify the specific targets of soy components; this has two aspects, namely the gene targets and the cellular targets. Genes whose expression levels are up- or downregulated with soy food intake are readily identifiable, given the availability, easy access to, and affordability of gene and proteome profiling tools. These analyses allow for the discovery of novel as well as the confirmation of previously identified, pathways that can serve as consistent biomarkers for favorable or poor tumor outcome. In studies from our group using Affymetrix GeneChip microarrays (Su et al. 2007b), we showed that expression of only a very low percentage of mammary epithelial cell transcripts (0.5 % of the total 14,000 genes evaluated) were altered with lifetime dietary exposure of rats to SPI or GEN. These sets of studies could be performed on tumors of breast cancer patients before and after specific drug interventions in the presence of soy (GEN) exposure to allow the identification of breast cancer signatures associated with soy therapeutic activity, in much the same way that an obesity signature for mammary tumors of 103 breast cancer patients was developed (Creighton et al. 2012). Such analyses could be followed-up with proteome profiling, using the same sets of tissues to confirm gain-or-loss-of-functional proteins associated with gene transcriptional changes. While complicated, the identification of which cell compartments are targeted by soy is imperative, given the increasing appreciation that the survival and recurrence rates in breast cancer are dependent on the stromal microenvironment (Polyak and Kalluri 2010; Conklin and Keely 2012). In this regard, our group has shown that adipocytes in the mammary stroma are targets of GEN. We demonstrated mammary adipocyte-specific genomic changes elicited by dietary exposure of rats to SPI *in vivo* that were recapitulated by GEN in the 3T3L1 adipocyte cell line *in vitro* (Su et al. 2009, 2011). Moreover, we showed the cooperative interactions between stromal-derived adipokine adiponectin and GEN to promote differentiation and enhance transcriptional response to estrogen receptor β signaling in mammary epithelial cells (Rahal and Simmen 2011). Consistent with these studies, numerous reports have correlated breast cancer survival with specific aspects of stromal biology (Conklin and Keely 2012).

The second challenge is to identify useful and consistent biomarkers to evaluate therapeutic efficacy. While patient complete response is the most obvious way to demonstrate efficacy, a systematic evaluation of biomarkers during the time course of treatment is useful for the monitoring of partial responses and can be of clinical benefit for prescribing drugs with negative side-effects at high doses. This would

require an understanding of the context of the biological response since expression of biomarkers likely differed with dose and duration of treatment; maybe defined by age, menopausal status and body mass index; and can be unexpectedly influenced by other components present in diets.

The third challenge is to determine which components of soy confer the best therapeutic potential. While isoflavones (predominantly GEN) are the best described and most studied among soy bioactive components, conflicting results obtained from preclinical, case-controlled, and limited phase 2 studies have lessened enthusiasm and support for further studies with isoflavones, using larger patient numbers. Findings that exposure to isoflavone-free soy diets was mammary tumor protective but those containing isoflavones were tumor-promoting in some studies (Martinez-Montemayor et al. 2010; Du et al. 2012), that soybeans contain proteins that are anti-cancer (Galvez et al. 2001; Jeong et al. 2007; Wang et al. 2008; Boué et al. 2009) and in particular mammary tumor-protective (Hsieh et al. 2010a, b), and that soy isoflavones act as weak antiestrogens, raising the potential for adverse effects on the reproductive system (Petrakis et al. 1996), make a compelling case for the testing of soy-associated components, other than isoflavones, for chemotherapeutic modalities.

Partial hydrolysis of the major protein component of soybeans yielded peptides with inhibitory effects on cancer cell lines *in vitro* (Wang et al. 2008; Mochizuki et al. 2009). A β-conglycinin derived peptide from the hydrolysate was found to inhibit growth of leukemia cells, alone and together with GEN (Wang et al. 2008). Saponin, another component of soy was also demonstrated to inhibit growth of human colon cancer cells (Tsai et al. 2010) and reduce colon tumor metastasis in mice, the latter by suppressing the expression of the matrix metalloproteinases (MMP)-2 and MMP-9 and stimulating the expression of tissue inhibitor of metalloproteinase-2 (TIMP-2) (Kang et al. 2008). In this study, mice fed the soybean component saponin prior to vein injection of colon cancer cells had reduced lung metastasis. While similar experiments using these molecules have not been conducted in breast cancer cells *in vitro* and in animal models of breast cancer *in vivo*, such studies demonstrate the potential of factors in soy foods that can selectively arrest tumor growth and metastasis.

Of recent interest is the soybean peptide lunasin, a 43-aa peptide component of post-translationally processed 2S albumin (Galvez and de Lumen 1999), which is also present in barley, wheat and other seeds (Jeong et al. 2010). Lunasin's anti-carcinogenic properties have been demonstrated in rodent models of skin (Galvez et al. 2001) and breast (Hsieh et al. 2010a) cancers and in colon and breast cancer cell lines (Dia and Mejia 2010; Hsieh et al. 2010b). In recent studies using non-malignant (mouse HC11) and malignant (human MCF-7) mammary epithelial cells, we showed that lunasin displayed common and distinct actions from those of GEN. In particular, lunasin induction of cellular apoptosis was mediated by PTEN, akin to GEN, albeit this occurred independent of p53, unlike that for GEN. Moreover, lunasin did not mimic GEN's inhibitory effects on the expansion of the limited cancer stem cell-like/progenitor cell population in MCF-7 cells (Pabona et al. 2013). The analyses of genomic signatures associated with lunasin signaling by

whole genome-array profiling and evaluation of whether serum levels of this peptide is associated with good prognosis in patients consuming soy foods will be required to begin to understand its clinical benefits.

5.7 Implications and Future Directions

Cancer remains a major global killer. Despite the seemingly positive report (http://www.cancer.org/Research/CancerFactsFigures/ACSPC-031941) that the annual rate of new cancer cases and the overall cancer death rate in the United States had dropped for the 10-year period between 1999 and 2008, the nation's health outlook remains problematic. The growing awareness of the association between obesity and cancer (Simmen and Simmen 2011), and the skyrocketing of the overweight and obese population, currently estimated at ~36 %, predict that this reduction in cancer cases will not be sustained, and that Americans (and by extension, globally) will be faced with higher cancer risks at adulthood. Thus, the impetus for dietary interventions for decreasing breast cancer susceptibility, beginning at early life, and improving outcome of breast cancer patients should be considered a priority rather than simply an option. While the use of soy foods is still a relatively under-appreciated treatment strategy, given the uncertainties regarding their role in mammary tumorigenesis, efforts by academicians in their respective laboratories and health care professionals in clinical settings should be enhanced to bring these new strategies to fruition. For academicians, the identification of the contextually-regulated environment wherein soy components can exert their most beneficial effects is crucial to maximizing their therapeutic potential. This is true not only for breast cancer but also for other cancer types like colorectal (Xiao et al. 2007, 2008; Yang et al. 2009; Yan et al. 2010) and prostate (Colli and Amling 2009) where the benefits of soy food intake have been reported but remain controversial (Adams et al. 2005). For clinicians, the task is to carefully screen for patients based on their contextual qualities with predicted favorable outcomes and determine at what point in therapy soy foods should be incorporated, as initial steps to determine its practical option and eventually as part of standard-of-care regimen.

The notion that soy food intake is a meaningful adjuvant strategy for conventional breast cancer therapies originally emerged from epidemiological reports that were subsequently evaluated by studies using animal models, leading to limited early phase clinical trials. Although soy isoflavones are considered the major targeting agents, they have not been conclusively associated with improved clinical outcomes. Given the complex system of the mammary environment, the recent discoveries pointing to the involvement of tumor-propagating cancer stem cells in programming breast cancer (Wicha et al. 2006; Damonte et al. 2008) might allow the streamlining of dietary effects directly to fetal and adult mammary stem cells to alter their behavior and inhibit neoplastic transformations, in the absence of confounding endocrine effects (Ablett et al. 2012). Based on the above, we propose a model wherein mammary stem/progenitor cells and when deregulated, cancer

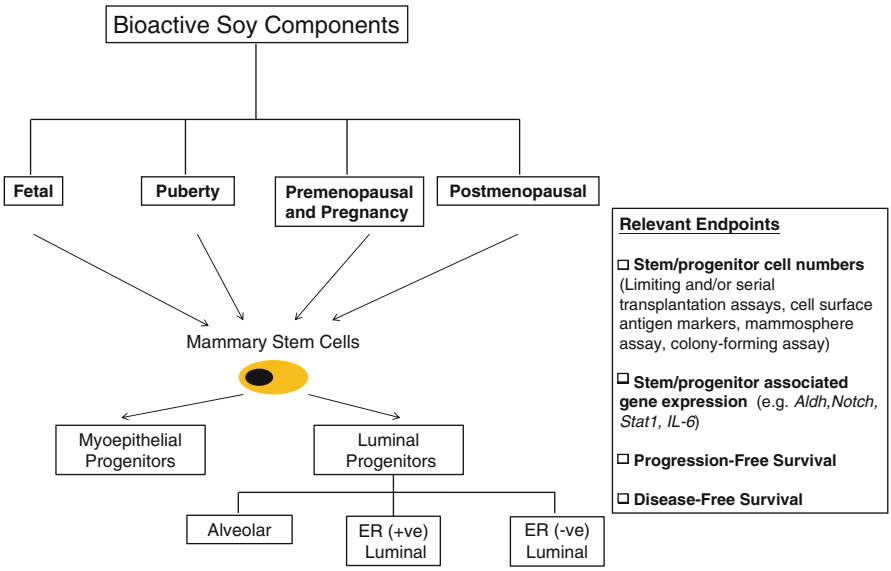

Fig. 5.3 A proposed model on mammary stem/progenitor cells as biological targets of soy food-associated bioactive components at various life stages. The actions of bioactive components on mammary stem and progenitor cells can be validated using relevant biological, molecular and survival endpoints in genetically engineered mouse models to inform future clinical trial designs and eventually, standard-of-care treatments. *Aldh* aldehyde dehydrogenase, *ER*(+) estrogen receptor-positive, *ER*(−) estrogen receptor-negative, *IL6* interleukin 6, *Stat1* signal transducers and activators of transcription-1

stem cells within the spectrum of a women's life (fetal stage, puberty, pregnancy, postmenopausal) may constitute targets of soy effects on immune/inflammatory, proliferation, and self-renewal processes (Fig. 5.3). The relevance of soy bioactive components in targeting mammary stem cells at different life stages could be initially tested using genetically engineered mouse models of breast cancer (Vaillant et al. 2008), which can recapitulate the distinct histopathological and molecular subtypes that characterize the human disease (Sorlie et al. 2001), to inform future clinical trial designs. While the paucity of tools to effectively target these cells remains a major challenge, this approach if successful could pave the way for novel therapeutic opportunities to eradicate cancer of the breast and other cancers.

In conclusion, it is apparent from multiple investigations cited here, that soy foods and soy isoflavone intake have the potential for becoming part of the standard-of-care treatments for breast cancer patients and survivors. A better understanding of their diverse effects under more defined and well-controlled clinical settings is warranted to yield definitive indications of the value of this strategy in the successful management of cancer.

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