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# **Plant Based Natural Products and Breast Cancer: Considering Multi-Faceted Disease Aspects, Past Successes, and Promising Future Interventions**

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Additional information is available at the end of the chapter

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## **1. Introduction**

### **1.1. Dealing with the burden**

World-wide, approximately 410,000 of the estimated 1 million females diagnosed annually with breast cancer will die from the disease [1]. According to Canadian Cancer Statistics, breast cancer continues to be the most commonly diagnosed cancer among Canadian women over the age of 20 (excluding non-melanoma skin cancer) and is the second leading cause of cancer deaths [2]. In 2012, it was estimated that 22,700 Canadian women would be diagnosed with breast cancer and 5,200 would succumb to the disease [2]. Furthermore, the recently published Prince Edward Island (PEI) Cancer Trends Report outlining cancer cases diagnosed from 1980 to 2009 indicates that in PEI, breast cancer is the most commonly diagnosed cancer in women [3]. While the overall incidence rate of breast cancer has increased marginally (0.5% per annum) and mortality rates have stabilized since 1992, the long-term survival in PEI women is significantly lower than the Canadian rate [3]. In summary, breast cancer continues to be a world-wide issue requiring attention. Increased awareness and advanced screening, resulting in early detection, is attributed to the increased incidence of the disease and such factors have led to a high percentage (approximately 95%) of all breast cancer patients being initially diagnosed with curable disease [4]. However, 30-40% of patients diagnosed with curable breast cancer succumb to disease reoccurrence [5]. Although increased diagnosis and advances in the treatment of breast cancer are clearly contributing factors in disease progression, eradication of residual malignancies and metastatic tumors via a systemic approach is considered the key

for success in treating cancer and increasing cancer patient survival [6]. There is an urgent need to explore agents that will be effective in preventing and treating metastasis of breast tumors.

## 1.2. The biology of breast cancer

In general, cancer occurs when a normal cell accumulates genetic and/or epigenetic changes caused by the activation or amplification of oncogenes and/or the mutation or loss of tumor suppressor function, resulting in the ability to proliferate indefinitely [7]. While these specific alterations lead to the transformation of the normal cell and partly determine the characterization of the tumor, the cell of origin and tumor (micro-) environment are also considered important factors contributing to tumor cell establishment, progression and therapeutic resistance [8].

## 2. Clinical characteristics and classification of breast cancer

### 2.1. Classifying breast cancer

Breast cancer classifications are largely explained by differences in tumor characteristics as determined by tumor appearance, histology, tumor marker expression (immunohistochemistry) or receptor status, and gene expression profiles. Alone, or in combination, these aspects can influence treatment, response and prognosis.

#### 2.1.1. Histology

Breast cancer represents many different histologies, however the majority (estimated to be more than 85%) of breast cancers are collectively derived from the epithelium lining in the ducts or lobes, and are classified as mammary ductal or lobular carcinoma [9]. Furthermore, this classification can be defined as *in situ* (meaning the proliferation of cancer cells within the epithelial tissue without invasion of the surrounding tissue) or *invasive* whereby the surrounding tissue is affected [9]. Included in the histological description of breast cancer is the status of invasion to the perineural and/or lymphovascular space and the presence of such is associated with more aggressive disease [10].

#### 2.1.2. Grade

Tumor grade is determined by comparing differentiation in normal and cancerous breast tissues. Normal cells in the breast become differentiated and acquire specific shapes and forms that reflect their function as part of the mammary system [9]. When cell division becomes uncontrolled, differentiation is lost. Breast cancers are either well differentiated (low grade), moderately differentiated (intermediate grade), or poorly differentiated (high grade) [9]. This progressive loss of features seen in normal breast cells is indicative of disease progression and poorly differentiated or high grade cancers have a worse prognosis than others [11].

### 2.1.3. Stage

Between 1943 and 1952, Pierre Denoix devised the TNM staging systems for all solid tumors to classify the progression of cancer [12]; the acronym can be explained by the fact that this model utilizes the size and extension of the primary tumor (T), its spread to the lymph nodes (N), and the presence of metastases (M). Although it is not utilized for all cancers (i.e.: brain and spinal cord cancer), the TNM system has progressed to the 7<sup>th</sup> textbook edition (TNM Classification of Malignant Tumors) [12] and remains the major system by which breast cancer is staged. An increase in stage number is based on larger tumor size, nodal spread (in particular, the sentinel node\*) and metastasis and is positively correlated with a worse prognosis [13, 14].

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\* Certain cancers spread in a predictable manner, from the site of origin to nearby lymph nodes (lymph glands) and then to other parts of the body. The very first draining lymph node is termed the "sentinel node" and is important in the sentinel lymph node biopsy (SLNB) technique utilized to stage the spread of certain types of cancer. The absence of cancer within the sentinel lymph node indicates that there is a high likelihood that the cancer has not spread to any other area of the body. Lymph node metastasis is considered one of the most important predictive signs in breast cancer, and thus can serve to guide the surgeon/oncologist to the appropriate therapy.

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## 2.2. Receptor status and gene expression profiles

Recent progress in molecular technologies has led to distinct breast cancer categories and five distinct tumor types and a normal breast-like group have been identified to date based on gene expression profiling [15], as summarized in Table 1.

Molecular Sub-type based on Gene Expression Profiling	Receptor Status based on Immunohistochemistry	Reference
Luminal A	ER+ve and/or PR+ve, Her2–ve, any CK5/6/Her1	[15-17]
Luminal B	ER+ve and/or PR+ve, Her2+ve, any CK5/6/Her1	[15-17]
Basal-like (triple –ive)	ER–ve, PR–ve, Her2–ve, CK5,6+ve and/or Her1+	[18]
Her-2+over-expressing (ERBB2)	ER–ve, PR–ve, Her2+ve, any CK5/6/Her1	[19]
Normal Breast –like	"unclassified" (negative for all 5 markers), displays putative-initiating stem cell phenotype	[20, 21]
"claudin-low" group	"unclassified" (negative for all 5 markers), displays putative-initiating stem cell phenotype, Often triple negative, displays low expression of cell-cell junction proteins and e-cadherin, frequently infiltration with lymphocytes	[20, 22]

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Positive (+ve), negative (–ve), estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptors (EGFR) 1 and 2 (Her 1 and 2) and cytokeratin 5, 6 (CK5,6).

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**Table 1.** Distinct Molecular Tumor Categories of Breast Cancer and their Receptor Status

However, most investigators use the presence (+) or absence (-) of immunohistochemical (IHC) markers or receptor combinations that are expressed by neoplastic cells. In this manner, distinct tumor categories to-date are identified by the expression of estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptors (EGFR) 1 and 2 (Her 1 and 2) and cytokeratin 5, 6 (CK5,6) [15-19, 23-25]. Generally, molecular sub-types correspond to IHC receptor status [26]. These subtypes are of great clinical and research importance as they are utilized to administer and target therapeutic regimes based on predictions of response. Furthermore, these subtypes have been shown to display a wide variety of responses to different treatments [27-30] and are associated with other clinical outcomes, such as patient relapse and overall survival. The most favorable outcomes are noted for the luminal A subtype, which are hormone sensitive [31]. The Her2+ and basal subtypes are noted as more aggressive and have fewer therapeutic options [31-33]. The normal-like and claudin-low are unclassified (negative for all major receptors), and associated with poor prognosis [20-22, 30, 34]. Several small studies support the concept that molecular subtype and tumor receptor status may change during ordinary disease progression and a major study completed by MacFarlane *et al.* in 2008 revealed that 21% of relapsed tumors had changes in either ER/PR or HER2 receptor status [35]. This significant proportion led the author to suggest that biopsies of relapsed/metastatic breast cancers should be performed routinely. This also should be an important consideration in research. In summary, treatment options considered effective in primary stage cancer may no longer be optimal in later stages of the disease, including relapses and metastasis as determined by the current classification scheme.

### 2.3. Other classification approaches

Other breast cancer classification approaches are also used to assist in both prognostic and treatment decisions. These include computer models that are based on a combination of several factors and offer individual survival predictions and calculations of treatment benefits [36]. For example, patients undergoing systemic adjuvant therapy can determine optimal treatment through the commercially available computer model *Adjuvant* which has been successfully validated in several cohorts, including the United States and Canada [36, 37]. Other useful classification tools utilized for breast cancer treatment choices include prognostic assessments (such as USC/Van Nuys prognostic index (VNPI)) and general comorbidity assessments [38, 39]. Also consider the case of familial breast cancer (genetic classification) whereby a patient may opt to undergo preventative measures such as mastectomy. Additionally, immunohistochemistry testing, other than those mentioned earlier, continue to prove favorable as prognostic markers across various molecular subtypes [40]. For example, in human breast cancer epithelial cell proliferation is considered a significant prognostic marker [41] and could possibly be used as a prediction tool to measure different hormone treatment related risks [42]. Therefore, the immunohistochemical marker, Ki67 (a nuclear protein expressed by cells in all active phases of the cycle except for quiescent or resting cells) is utilized frequently to evaluate proliferation [43]. Such labeling indicates a significant association with higher carcinoma grade, clinical response to endocrine therapy, higher risk of relapse, and worse survival in patients with early breast cancer [44].

### 3. Cell of origin

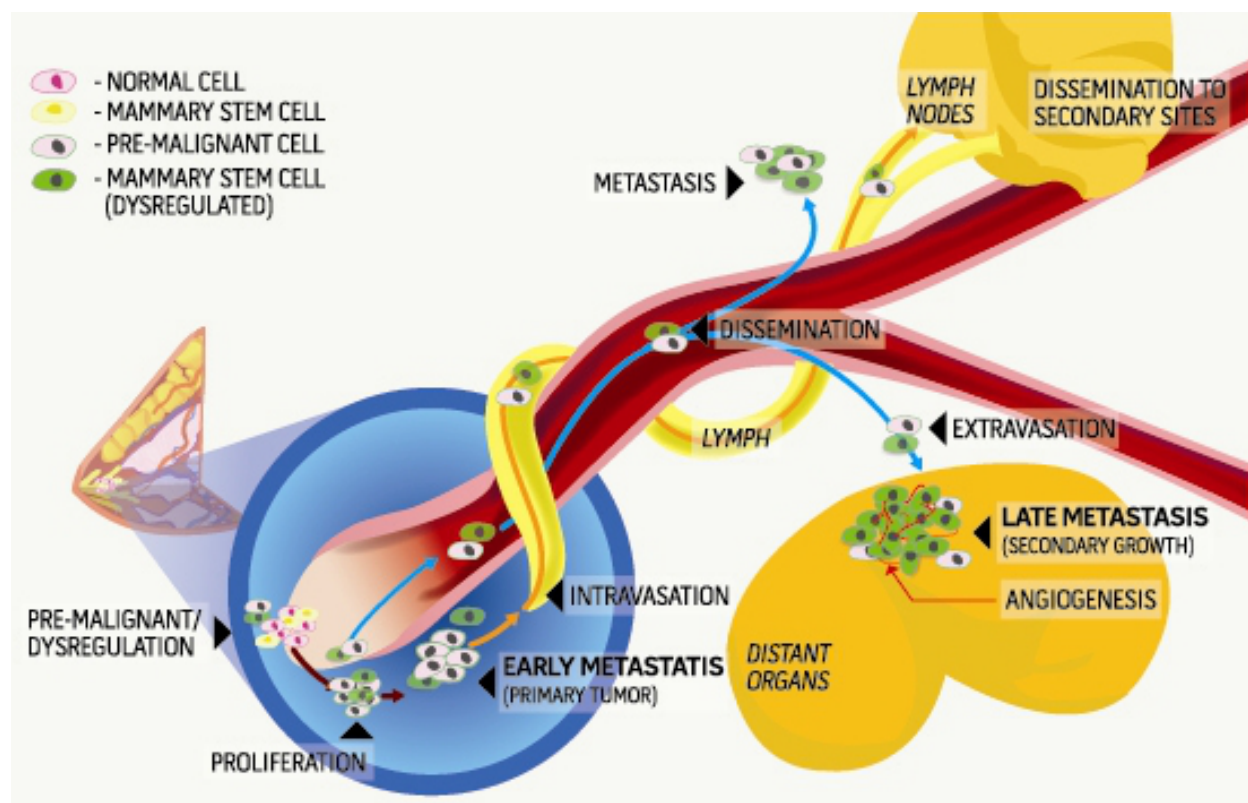
While scientists have predicted that specific breast cancer types may arise from different types of progenitor cells, it has been difficult to identify the cells of origin, and the topic remains controversial to date. Breast tumors represent a heterogeneous collection of cell populations with different biological properties [45]. Shared molecular features have made it difficult to distinguish the cell populations of breast cancer tumors and only recently have researchers been able to differentiate stem cells from other progenitor cells. Within this context, tumor evolution has been explained by two main theories. The traditional clonal evolution model is based on the premise that all tumor cells have the capacity to undergo self-renewal which is an indication of their potential to undergo tumor progression and drug resistance [46, 47]. The Cancer Stem Cell (CSC) theory emphasizes the ability of only a minor population of tumorigenic cells capable of self-renewal and differentiation [20, 48] and this specific sub-set of CSC's gives rise to new tumors which are phenotypically identical to the original tumors [49]. CSC's are believed to be a small population of cells with dysregulated self-renewal properties capable of continuous self-renewal and differentiation and responsible for tumor existence treatment resistance and relapse. Existence of CSC's in various types of tumours, including breast cancer [50],[51] has been identified. ALDH (aldehyde dehydrogenase)-1 is a marker of normal and malignant human mammary stem cells [52], and these cells can also be isolated using the cell surface markers epithelial-specific antigen (ESA), CD44 and the absence of the expression of CD24 [53]. When transformed cells undergo epithelial-mesenchymal transition (EMT), they have been noted to gain properties of stem cells [54]. Although evidence supporting the CSC model was initially obtained from acute myeloid leukemia [55], successive studies maintain that solid tumors, including breast cancer tumors, are also driven and sustained by CSCs [50].

Regardless of origin, an abundance of research has clearly confirmed the existence of cancer stem cells (CSCs) or tumor-initiating cells (TICs) in a variety of human cancers [55-58], including breast cancer [50]. Nonetheless, most of the therapeutic approaches available, inclusive of chemotherapy and radiation, lack the ability to effectively kill these populations [59-62]. This may explain the lack of progress in eliminating cancerous tumors and preventing metastasis and may help to rationalize therapeutic resistance; therefore, the CSC or TIC population has become a target for cancer prevention and therapy [63]. A general consensus exists in the literature that breast cancer re-occurrence is assumed to be caused by a sub-population of tumor initiating cells possessing stem cell attributes of a tumor as well as resistance to chemotherapy, radiation and other forms of treatment [64-66]. Of great interest is the role of CSC's in tumor relapse and resistance to therapy and recent articles suggesting that such resistance can be overcome.

### 4. Breast cancer progression models

The combined research efforts of various scientific disciplines have resulted in the development of a disease progression model for breast cancer (See Figure 1) and include a continuum of lesions through to invasive carcinoma and eventually metastatic disease [30, 48, 67, 68]. For decades, it was thought that metastatic dissemination occurred as a final step in cancer and

was the responsibility of genetic changes of malignant cells in the primary tumor [69]. In 2008, Husemann *et al*, used transgenic mice to show systemic dissemination (specifically to lungs and bone marrow) of mammary tissue derived premalignant cells prior to the emergence of mammary tumors [70]. Additionally, this research reported that systemic dissemination of tumor cells can occur in pre-invasive stages of tumor progression as observed in female patients with ductal carcinoma *in situ*. A complementary accumulation of evidence supports the evolution of an early dissemination model, where malignant cells outside the primary lesion can also migrate to distal sites (such as lung and bone marrow) and cause tumors via various genetic programs [54, 71, 72]. Such a model is inclusive to “self-seeding”, the term coined when cancer cells not only seeds regional (lymph nodes) and distant sites but also fuel the growth of the original tumor itself [73].



**Figure 1. (Tobin, GA, 2011) Physiological Aspects of Disease Progression in Breast Cancer:** The development of breast cancer has been proposed as a multi-step process. The most deadly aspect of breast cancer is metastasis and involves a cascade of reactions. Although the molecular mechanisms underlying this process are not fully understood, any disruption along the cascade could arrest disease progression.

Thus, breast cancer is not a single disease, but rather an assortment of diseases with diverse characteristics and clinical outcomes which will likely always require a variety and/or combination of treatments or alternatively, a broad spectrum application. Combine this with the fact that, despite major advances in our understanding of the biology of cancer, further research is required to improve our understanding of tumor establishment, progression and dissemination - the principal cause of mortality. Then, our goal in breast cancer research

regarding treatment would be to identify novel therapies and maximize, based on our current understanding of the disease, the use of such in preventing and treating as many aspects of the disease as possible.

## 5. Molecular and endocrine controls

### 5.1. BRCA and other gene expression signatures associated with increased risk of breast cancer

Less time, low cost and new sequencing technologies are all accomplishments that yield impact on molecular biomedical research. Recently, for example, such advances (combined with some prospective epidemiology studies) have allowed researchers to compare the DNA from healthy breast tissue, initial tumor cells and then cells obtained nine years later when the breast cancer had metastasized [74]. The 32 DNA mutations reported in the metastasized cells are a prime example of how technological advances can serve to provide us with ample data regarding gene expression that may offer insights into the progression of breast cancer disease. However, consider that gene expression profiling / signatures of primary breast tumors can only be used as a predictor of susceptibility or disease progression in breast cancer, particularly metastasis. Currently, it is not possible to accurately predict the risk of metastasis or prevent it. As a result, more than 1/2 of the patients treated with adjuvant chemotherapy are needlessly exposed to harmful side effects [5]. This presents another compelling reason to further study drug targets that are specific to, and have potential for, treatment in metastatic breast cancer.

Nonetheless, based on current knowledge, and aside from the many identified factors that could impact the risk of developing breast cancer (including personal and environmental), genetic mutations in critical cancer genes (both tumor suppressor and oncogenes) have been identified for their increased or associated risk with breast cancer [75]. Of the many that have been reported throughout the history of cancer genetics, Table 2 below captures those that stand out principally for their research and/or clinical significance in relation to breast cancer and summarizes major function, encoded proteins and known disease associations or risk factors. Such genetic aberrations are acquired over a person's lifetime; or less commonly, are inherited. While it is estimated that only 5% to 10% of breast cancers are hereditary, some gene variations are associated with both hereditary and somatic mutations [75]. The tumor suppressor genes *BRCA1* and *BRCA2* are the major genes related to hereditary breast cancer [76], and mutations in these and other *BRCA* genes in women are associated with a 60-80% risk of developing breast cancer throughout their life span [77]. Other genes with inherited alterations, including *CDH1*, *PTEN*, *TP53*, *CHEK 2*, and *ATM* have been noted to increase or are associated with the risk of developing breast cancer [78]. Most notably, the latter three have presented the strongest evidence related to the risk of developing breast cancer [79]. Somatic mutations mostly reference *ERBB2/HER2(neu)* in breast cancer, however *TP53* genes and others have been associated with some cases of breast cancer in this manner [80]. It is noteworthy that not all people who inherit mutations in these genes will develop cancer.



Gene	Major Function: Associated Proteins	Risk Factor	Ref.
BRCA1 (BRCA1 Cancer gene one)	Encodes breast cancer type 1 susceptibility protein; responsible for DNA repair, transcriptional regulation and cell cycle check point control.	Strong evidence indicating a 60 – 80% risk of developing breast cancer for people with mutations.	[81 - 86]
BRCA2 (BRCA2 Cancer gene two)	Encodes BRCA2 susceptibility protein involved in the repair of chromosomal damage, especially in the error-free repair of DNA double strand breaks. As with BRCA1, indicates a high degree of risk.	Reduced levels of the BRCA2 protein may cause Fanconi anemia. Patients with such are prone to several types of cancers, including reproductive system associated tumors.	[87] [88]
ATM (Ataxia telangiectasia mutation)	Encodes protein with a phosphatidylinositol 3-kinase (PI3K)-like domain which plays a central role in the complex processes that repair DNA double-strand breaks. Also involved in regulation of cell cycle progression and the maintenance of genomic stability.	Confers susceptibility and linked to an modest increase (up to 2 times) of breast cancer.	[89-93]
p53 (TP53) (Tumor Protein p53)	Encodes p53 protein and regulates cell cycle, preventing tumor growth.	Causes Li-Fraumeni syndrome: results in higher-than-average-risk of breast cancer and several other cancers.	[94-96]
CHEK2 (Checkpoint kinase 2)	Encodes a serine/threonine-protein kinase which plays a critical role in DNA damage signaling pathways. Phosphorylates and regulates the functions of p53 and BRCA1.	Causes Li-Fraumeni syndrome. Can double breast cancer risk.	[97-99]
PTEN (Phosphatase and tensin homolog)	Encodes phosphatase and tensin homolog protein, namely phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase, which is involved in the regulation of the cell cycle.	Causes Cowden syndrome which presents a higher risk of both benign and cancerous tumors in the breast, digestive tract, thyroid, uterus, and ovaries.	[100, 101]
CDH1 (Cadherin 1 Gene) or E- cadherin (epithelial cadherin)	Encodes E-cadherin protein. Down-regulation decreases the strength of cellular adhesion, increases cellular motility; allowing cell invasion.	Increased risk of breast cancer, particularly invasive lobular breast cancer.	[102-104]
** ERBB2, (HER2/neu), or VEGF	Encodes a transmembrane receptor with constitutive tyrosine-kinase activity.	Amplification occurs in 15-30% of human breast cancers.	[34] [80]

\*\* ERBB2 (erythroblastosis oncogene B), HER2/neu (Human Epidermal growth factor Receptor2, neu: derived from a rodent (neu)ral tumor), VEGF (vascular endothelial growth factor).

**Table 2.** Familiar Genetic Mutations, Encoding Proteins and their Major Functions in Relation to the Associated and Increased Risk of Developing Breast Cancer.

Those listed in Table 2, as mentioned, represent familiar genes associated with breast cancer. However, others genes that are applicable in breast cancer progression will be reviewed in terms of their associated cell signaling pathways. Considering the profound implications that the CSC theory has for cancer chemoprevention and therapy, combined with our interest in plant based molecules, we will also examine gene function in the context of opportunities for natural product compounds in CSC self-renewal.

## 5.2. Endocrine controls

Over the years, and more notably since the discovery of suitable breast cancer cell lines and animal models, the symbiotic relationship between lab research and clinical investigations have advanced our knowledge of endocrine action. Breast cancer is influenced and highly regulated by several sex and growth hormones (including estrogens, androgens, progesterone, prolactin and insulin-like growth factors) and each of the sub-types and gene expression patterns of breast cancer are characterized by both unique and specific endocrine controls [105]. Particularly, estrogens and progesterone have received the greatest attention likely because of their involvement in normal and neoplastic mammary tissue and the scale of their associated risk estimation for breast cancer. Research into the role of androgens has likely been a consideration in breast cancer research as androgens are necessary precursors to all endogenous estrogens. The role of prolactin in the pathogenesis of breast cancer remains unclear given the scarcity of studies to date. Similarly, although well studied, the role of Insulin-like growth factor (IGF) in breast cancer is inconsistent.

There are challenges in defining the role of progesterone in breast cancer and the role of progesterone receptor (PR) action in breast cancer remains divisive. In breast cancer, progesterone has biphasic effects (both proliferative and inhibitory) on breast cancer cell lines grown *in vitro* [106, 107]. Depending upon cellular context and/or the presence of secondary agents, there may be a role for progesterone as a priming agent with growth promoting activity [106].

While progesterone is found as a single hormone, the major endogenous estrogens in females include estrone (E1), estradiol (E2), and estriol (E3) and are primarily produced during menopause, in non-pregnant and pregnant females, respectively [108]. A variety of synthetic (xenoestrogens) and natural substances have been identified that also possess estrogenic activity including those derived from plant products (phytoestrogens) and fungi (mycoestrogens) [108]. The actions of estrogens are mediated by their respective receptors binding to specific DNA sequences to activate the transcription of estrogen receptor (ER)-regulated genes, including direct target genes [109]. Approximately 80% of breast cancers demonstrate expression of PR and/or ER [110]. Once established, these breast cancers which are classified as either hormone-sensitive or hormone-receptor-positive cancers are reliant on hormones to grow. Thus, treatment includes the suppression of hormone production in the body for these specific breast cancers. Therefore, this section will provide an overview of estrogens, their past indications in the treatment of breast cancer and their potential role in relation to naturally occurring dietary compounds.

### 5.2.1. *The role of estrogens and breast cancer*

The link involving estrogen and breast cancer can be traced back to as early as 1896 when a surgeon in England reported an improvement in condition in three young female breast cancer patients after the removal of their ovaries [111]. Since estrogen had not yet been discovered, the surgeon had unknowingly removed the source of estrogen that promotes the survival and division of cancer cells [111]. Since that time, a lot of evidence has shown that interactions between estrogens and their receptors influence the pathogenesis of breast cancer. Estrogen promotes proliferative effects on cultured human breast cancer cells [112]. Estradiol affects breast cancer risk by controlling the mitotic rate of breast epithelial cells and high levels of estradiol in post-menopausal women are also known to increase the risk of breast cancer [113]. Estradiol has also been shown to increase breast cancer risk via its metabolite, catechol estrogen 4-hydroxyestradiol, causing direct DNA damage through the formation of free radicals [114]. Estradiol has also been shown to modulate breast cancer cell apoptosis [115]. ER is a major determinant of the cellular response of estrogen and has been indicated in breast cancer promotion [116, 117]. The binding of estrogen to the ER modulates the transcription of a series of genes, including those coding for proliferation.

The close relationship between the etiology of breast cancer and exposure to estrogen warrants examination of key variables that may affect estrogen homeostasis, in particular those exhibiting anti-estrogen activity. Because of their impact on the primary and metastatic aspects of disease, anti-hormonal drugs are the mainstay breast cancer treatment. The goal in treating hormone receptor +ve breast cancers is to utilize drugs which suppress production of estrogen in the body. Estrogens in naturally occurring dietary compounds such as soy are used as an alternative to hormone therapy because of their anti-proliferative effects. This practice, in breast cancer treatment, is widely known as hormonal therapy, or anti-estrogen therapy, but is not representative of the term hormone replacement therapy [118].

## 6. *In vitro* and *in vivo* breast cancer models and their relevance to human disease

In 2007, Vargo-Gogola and Rosen summarized appropriately, within their title, the challenge of modelling breast cancer; "One size does not fit all"[31]. Considering the heterogeneity nature of this disease, these researchers rather addressed the reasonable question of the most powerful way to investigate breast cancer with respect to cell lines and animal models. We agree with their conclusion that an integrated and multi-systems approach is the strongest way to model this disease. Here, we briefly discuss the most commonly utilized *in vivo* and *in vitro* models and their relevance in human breast cancer.

### 6.1. Mammary carcinoma cell lines

Our current knowledge of breast cancer is mainly based on *in vivo* and *in vitro* studies completed with breast cancer cell (BCC) lines. The first BCC line, namely BT-20, was established in 1958 [119] and since then the number of permanent lines sustained are relatively low despite continuous research in breast cancer [120]. In fact, the number of commercially available BCC

lines (ranging from 70 – 80 based on ATCC and similar commercial suppliers) represent mostly cell lines that were established more than 25 years ago. It has been suggested that this inefficiency in producing new and improved lines is likely due to technical difficulties in extracting viable tumor cells from their neighboring stroma [120]. In the past, it has been estimated that more than two-thirds of all scientific abstracts related to studies on mentioned BCC lines include the MCF-7, T-47D, and MDA-MB-231 lines [120]. Although no single cell line is entirely representative of human breast cancer, cell lines have been widely used. As a result, we have a greater understanding of breast cancer biology. Additionally, these cell lines have advanced the ability of pre-clinical models to predict pharmaceutical activity and therapeutic applications for further study. Despite this, the use of cell lines for application in human disease is limited. The single most significant question, considering the devastating effects or outcomes of metastasis, is the issue of whether these cell lines are representative of metastatic conditions, or contain tumor initiating subpopulations. There are studies to indicate distinctions between tumor and non-tumor initiating populations at the cellular and molecular level [31, 121]. Research indicates that the two luminal subsets (A and B) evident in tumors are not apparent in the cell lines, and the basal-like cell lines are actually representative of two distinctive clusters (A and B) that are not apparent in analyses of primary tumors [16]. The differences in cell culture may be due to the absence of stromal or physiological interactions and/or signaling [122] [123]. However, there is evidence that cell lines may be derived from subpopulations of tumor cells that are selected because they grow well. For example, it has been noted that differences between the genome aberration patterns for the basal-like and luminal clusters in the cell line system don't match differences in these subtypes in primary tumors. Furthermore, the highly invasive Basal B cells carry the distinctive phenotype associated with the subpopulation of tumorigenic stem cells identified in breast cancer [50, 124]. Also, one must consider the issue of variants of the same cell lines and the phenotypic changes that can occur due to environmental exposures, rate of passage and age of cells.

Perhaps the most highly debated topic regarding the use of breast cancer cell lines revolves around the MDA-MB-435 cell line, and by extension MDA-MB-435S (ATCC® HTB-129™) and derivatives. Spontaneously metastatic and originally designated as Basal B and being derived from a breast carcinoma, the MDA-MB-435 cell line has subsequently been questioned because of recent evidence indicating that it might originate from melanoma or may have been cross-contaminated with the M14 melanoma cell line [125-130]. While some researchers continue to use this cell line as a breast cancer mouse model [131] and conclude that this cell line is in fact breast cancer cells [132], others insist that the MDA-MB-425 cell line is a melanoma cell line and renders its use as improper in breast cancer studies [133]. Later in the chapter, we will describe some research that has been conducted using MDA-MB435 cell lines and/or its derivatives as breast cancer models in research involving natural, despite this controversy. This may not be all bad news for natural product researchers. On the bright side, natural product therapies indicated in the controversial cells line MDA-MB-435 and its derivatives then point toward activity in melanoma as opposed to breast cancer.

Nonetheless, there is clearly a need for new cell lines that are representative of all the known sub-types of breast cancer. Currently, researchers have the option of using a group (or panel)

of subtype cell lines to increase power in their research, as proposed by Neve [2006] [129]. To date, and based on our knowledge, there has been no evidence of a male breast cancer derived cell line. With the incidence of male breast cancer rising [2], relevant models require further development.

Cell lines are easy to culture, inexpensive in comparison to animal models, and provide an unlimited source of homogenous material to work with. Comparisons between researcher and studies are usually fairly consistent and reproducible. A limitation of cell cultures is their inability to measure tumor-stromal cell interactions, however the growth of cell lines for transplantation allows for such *in vivo*.

## 6.2. Animal models

Animal models have allowed researchers to gain much insight into the disease progression, in particular metastasis. In addition to advancing the concept of metastasis [134, 135], our knowledge of the biological function of genes and signaling pathways has progressed as a result of the vast amount of information generated from animal models. Experimental systems based on mouse models that are currently used to study breast cancer can be categorized as tumor transplantation and genetically engineered mice (GEM) – often termed as transgenic. Here we will focus on commonly utilized models within each of these categories and discuss some of the advantages and limitations of each in relation to human breast cancer disease relevance.

### 6.2.1. Transgenic or (GEM) models

Different GEM breast cancer models are useful for studying distinct signaling interactions and for testing therapies that target pathways involved. Genomic deletion of tumor suppressor genes or the transgenic insertion of oncogenes allow these mice to be a relevant tool to investigate the spontaneous initiation of breast tumors in each step of metastasis [136]. Specific to breast cancer, the expression of oncogenes in explicit breast regions are restricted via a mammary gland specific promoter [136]. MMTV (mouse mammary tumor virus) or WAP (Whey Acidic Protein) promoter control the expression of oncogenes (i.e: PyMT, ErbB2, Wnt1, or Ras) in transgenic mice and initiate mammary gland tumors which lead to metastasis in various organs [137]. The MMTV-PyMT transgenic mouse model represents hyperplasia, adenoma, and early or late carcinoma as seen in human cancer stages. In breast cancer, this model demonstrates short latency tumor development specific to the mammary gland with a high incidence pulmonary metastasis [138]. The deletion of mammary epithelial cell-specific tumor suppressor genes results in similar conditions observed in human cancer patients, including spontaneous tumors, bone metastasis, loss of estrogen receptor (ER) expression, and hormone-responsiveness [139, 140]. Crossing GEM mice with other transgenic mice has allowed researchers to investigate the role of genes and signaling pathways in tumorigenesis and metastasis. While tumor progression was delayed in MMTV-PyMT or MMTV-ErbB2/neu mice crossed into an Akt or PTP1B-deleted genetic background [141, 142], increased metastasis was observed in the MMTV-PyMT mouse in a CD44<sub>-/-</sub> background; the later indicates the importance of receptors and other secreted factors in epithelial–stromal interactions or overall

tumor environment [139]. In this regard, much insight has been gained through the use of GEM models to indicate the role of both innate and adaptive immune responses in the progression of breast cancer. For example, pulmonary metastasis is notably diminished via the selective loss of the interleukin (IL-) 4 cytokine in PyMT/IL-4<sup>-/-</sup> mice through inhibition of IL-4 mediated EGFR signaling of mammary tumors [143]. The understanding of such interactions are important if we consider that tumor-associated immune cells release numerous growth factors, including cytokines, chemokines, and enzymes that promote tumor growth, angiogenesis, and metastasis, and thus are associated with poor prognosis [136]. Inducible GEM mouse models (those capable of expression or repression of certain genes) have advanced knowledge with respect to the association of multiple genetic mutations of oncogenes in relation to tumor establishment and maintenance [144]. There are several drawbacks to consider in the use of GEM models. There are inherent differences in mouse and human, for example: BRCA1 and p53 are on the same chromosome in mice, but not in humans [136, 144]. Technical discrepancies, depending on methods used to generate the model and the interpretation of results can present varying outcomes. Also, temporal and spatial tumor development and metastasis differ depending on the mouse strain used. For example the percentage of ER/progesterone receptor (PR)+ve tumors in Wap-Cre/Trp53<sup>-/-</sup> mice are high while MMTV-Cre/Trp53<sup>-/-</sup> mice report a low incidence of such [145]. The molecular profile of mammary tumors from GEM models represent the subtypes of human breast cancers, including those included in Table 1, however no single model to date is representative of all expression patterns and characteristics of human cancer.

#### 6.2.2. Tumor transplantation model

Syngeneic transplantation refers to the transfer of cancer cells from one mouse into another with identical genetic background [146] and such models are used to establish organ specific metastasis [147]. The 4T1 breast cancer tumor model (a syngeneic mouse model) is considered excellent for testing experimental cell-based immunotherapy strategies and is comparable with stage IV human breast cancer [148]. The injection of 4T1 cells, originally derived from a spontaneous mouse mammary tumor are injected into the mammary fat pad of a syngeneic animal and the rapid proliferation of cells forms tumors and eventually metastasizes to the lungs, liver, bone and brain [146, 147, 149]. Furthermore, this model has been refined through the development of 4T1 cell lines to employ varying degrees of metastasis that are location specific and representative of distinct gene expression signatures [150]. Xenograft transplantation encompasses the use of human cancer cells into immuno-compromised mice via intravenous, intraperitoneal or subcutaneous injection, orthotopically or ectopically [136, 151]. First discovered in 1962, nude mice have been commonly used for xenotransplantation because they lack a thymus and are unable to mount most types of immune responses, including rejection of allografts and xenografts [152]. Severe Combined Immunodeficiency (SCID) mice present with impaired ability to make T or B lymphocytes and are used as model organisms for research into transplantation strategies as they cannot reject tumors and transplants [153]. In general, rodents with immunological defects are typically resistant to growth of mammary carcinomas, and despite improvements these approaches have yielded low percentage of successful breast tumor engraftment compared to other types of cancers

[154]. Recent mouse models have contributed significantly to the understanding of breast cancer, however further research into suitable animal models will be required to advance development of new therapies for breast cancer. The growth and metastasis of human breast cancer cell lines *in vivo* allows the measurement of gene function relative to disease progression and has provided much insight into the use of investigational drugs intended interrupt or interfere with tumor growth [155].

Table 3 provides an overview of the defining characteristics of established breast cancer cell lines (mouse and human) commonly used in both *in vitro* and transplantation models.

Cell line	Species	ER Status	PR Status	Metastasis Location	Ref.
4T1	Mouse	+	+	Lymph node, blood, liver, lung, brain, bone	[149]
BT-474	Human	+	+	Bone	[156]
FII3	Mouse	+	+	Lung	[157, 158]
MCF-7	Human	+	+	Lymph node, lymphatic vessel	[159, 160, 161]
MDA-MB-231	Human	-	-	Lung, liver, brain and bone	[162, 163]
MDA-MB-435	Human	-	-	Lung	[164]
MDA-MB-453	Human	-	-	Bone	[165]
SUM1315	Human	-	-	Lung, bone	[166, 167]
SUM149	Human	-	-	Lung	[167]
T47D	Human	+	+	Lymph node, lymphatic vessel	[168]

**Table 3.** Breast Cancer Cell Lines for *in vitro* and transplantation models

It is unlikely that any one transplantation model will ever replicate the complexity of the whole cancer process; however studies to date demonstrate that xenographs are relevant in human breast cancer. Treatment with Herceptin was shown to improve the anti-tumor activity of paclitaxel and doxorubicin against HER2/neu-overexpressing human breast cancer xenografts leading to consecutive favorable clinical trials [169, 170]. Davis *et al.* [2004] reported the effective inhibition of tumor growth and metastasis in an orthotopic xenograft model by the use of combination therapy of paclitaxel and neutralizing antibodies targeting vascular endothelial growth factor receptor 2 (VEGFR2) [171]. The results of this research likely led to the development of bevacizumab, a humanized monoclonal antibody that targets vascular endothelial growth factor A (VEGF-A) [172]. Although, bevacizumab was removed as a breast cancer indication by the FDA [173], this is yet another example of how transplantation models lead to further development in the clinic. While these are merely a few cases for breast cancer and the use of xenograft studies, the information obtained from such has been translated into successful clinical trials for a variety of cancers [174-178]. Furthermore, useful information has been gained from transplantation models with respect to toxicity and in the identification of predictive biomarkers.

In spite of these successes, the xenograft model presents disadvantages in its ability to predict clinical response to therapy. Most animal tumors do not accurately model clinical metastatic disease. The use of immuno-compromised mice prevents an immune rejection response which is fundamentally different from the human system where the immune response promotes primary growth of tumor cells and their migration to secondary organs [136, 179]. Most xenograft models do not metastasize at the common sites of human breast cancer (such as lymph nodes, liver, bone and brain) as they prefer to colonize in the lungs [5]. However, improved models that have been created to represent human breast cancer metastasis from a primary orthotopic site to human bone, such as initially published in 2005 by Kuperwasser *et al* [167]. Consider that some xenograft models which utilize subcutaneous injection of tumor cells into the flank and mammary fat pad are not as representative of clinical disease as those that use orthotopic transplantation of cells into the mammary gland. Inherent discrepancies in the background of mice and humans should be considered, and this is of particular importance in predicting side effects for cancer therapy targets [180]. Additionally, the area of metastasis can change depending on the cells line utilized or methods of inoculation [167]; thus, there are considerable technical issues. One could overcome such restrictions by use of clinical isolates. However the utility of primary xenografts is inefficient; access is limited, results are restricted by sample size and thus, studies to date indicate only some degree of success [181]. Current research with aims to generate partial human immune systems or intact populations of human cells in mice systems may also overcome such limitations. We must consider that despite all advances to date, animals do not represent a complete model of human disease. For example, tumor relapse would be especially problematic to study as the usual life span in the majority of mice does not exceed two years. Aside from these underlying basic issues, there have been issues associated with the testing of natural products within these models. Further clarity on the fundamental mechanisms of tumor progression and metastasis and new drug targets will be unveiled as mouse models advance. Until then, we will continue to rely on current disease models and choose such based on disease state and therapeutic targets.

## 7. Breast cancer signaling pathways

Each of the identified breast cancer subtypes and gene expression patterns are dependent on different oncogenic pathways [105, 182]. The maintenance and differentiation of normal breast tissue is controlled by many signaling pathways and involves cytokines and chemokines, growth factors, steroid hormones, integrins, adhesion molecules and their respective receptors [183]. The regulation of such by single or combined components of the tumor microenvironment (such as fibroblasts, macrophages / lymphocytes, endothelial cells, vessels and proteins of the extracellular matrix (ECM) and stroma) have been implicated in various ways in the promotion, growth invasion and metastasis of breast cancer [184]. Cross talk or communication between the cancer cells and the factors within the tumor environment, including secretion factors from the tumor itself, can modify expression and signaling [184]. Of the several pathways indicated to play a role in cancer and CSC self-renewal, Notch, Wnt/Beta( $\beta$ )-catenin,



and Hedgehog (Hh) have been identified in human mammary cancer [124, 185, 186]. Additionally, evidence has mounted to strengthen the link between nuclear factor kappa-B (NF- $\kappa$ B), stem cells and breast cancer as elegantly reviewed in a recent paper by Shostak and Chariot (2011) [187].

### 7.1. Notch pathway

Four Notch proteins, namely Notch-1 to Notch-4, are expressed as transmembrane receptors in a variety of stem/progenitor cells [188, 189]. The binding of specific surface-bound ligands are responsible for triggering cleavage events at the Notch proteins by ADAM (A Disintegrin and metalloproteinase domain-containing protein) protease family and  $\gamma$ -secretase [188-191] causing the intracellular domain of Notch to be released and translocate to the nucleus. Once in the nucleus, downstream target genes (including c-Myc, cyclin D1, p21, NF- $\kappa$ B) are activated [190, 192-197]. Known for their ability to modulate the development of various organs and control cell proliferation [198], the notch activated genes and pathways have been reported to drive tumor control through the expansion of CSCs [198-202]. This associated role in self-renewal function of malignant breast cancers CSCs [198], combined with the fact that Notch inhibitors can kill breast cancer cells *in vitro* and *in vivo*, may partially explain why Notch expression and activation has been associated with a poor prognosis in mammary carcinomas [203-205]. In fact, research findings in breast cancer have presented compelling reasons to target Notch as a therapeutic target in solid tumors. In addition to its ability to regulate survival and proliferation in bulk cancer cells [205] and CSCs [206-209], notch plays a pro-angiogenic role in tumor endothelial cells [210, 211]. Farnie *et al* reported that activated Notch-1, Notch-4, and Notch target Her-1 expression in ductal carcinoma mammospheres *in situ* samples, but not from normal breast tissue [206, 212, 213]. Inhibition of Notch with a gamma-secretase inhibitor (GSI) or a neutralizing Notch-4 antibody has been reported to reduce the ability of ductal carcinoma in situ-derived cells to form mammospheres [207, 214]. Such results suggest that Notch inhibition may have significant therapeutic effects in primary lesions, may be able to preferentially target breast CSCs (responsible for reoccurrence and metastatic disease) and counteract angiogenesis [213]. Cross talk with the NF- $\kappa$ B pathway and Notch1 have been reported in a variety of cell interactions [192, 215-219], including the stimulation of NF- $\kappa$ B promoters [217] and the expression of several NF- $\kappa$ B subunits [192, 215-220].

### 7.2. Wnt/ $\beta$ -catenin pathway

The canonical (Wnt/ $\beta$ -catenin) pathway, including Wnt-1, -3A and -8 is likely the best characterized and traditionally defines Wnt signaling, however other pathways have been described including a non-canonical (planar cell polarity) pathway (including Wnt-5A, -11) and the Wnt/Ca<sup>2+</sup> pathway (protein kinase A pathway) [221-224]. Although it has been more than 25 years since the discovery of the Wnt gene, its structure remains unknown and signaling pathways are not well defined, especially those independent of  $\beta$ -catenin. Perhaps this challenge can be somewhat explained by the recent discovery that differences in cell signaling outcomes may be attributable to precise pairings of Wnt ligands with analogous cellular receptors [225]. For example, if we consider that the mammalian genome codes for 19 Wnt proteins and 10 Fzd

receptors, there are potentially 190 Wnt/Fzd pairing combinations [226]. Although all of these ligand/receptor pairings have not been unveiled, we already know that the Wnt/ $\beta$ -catenin pathway has been established for its ability to alter cell proliferation, migration, apoptosis, differentiation and stem cell self-renewal [224, 227-230]. The essential mediator of the canonical pathway is  $\beta$ -catenin, and its two known distinct functions are based on cell specific locations. Accumulation of  $\beta$ -catenin within the cytoplasm leads to activation of Wnt target genes such as c-Jun, c-Myc, fibronectin and cyclin D1 [186, 231-236]. Prior to nuclear translocation,  $\beta$ -catenin operates in the membrane to maintain cell-cell adhesion via cooperation with the epithelial cell-cell adhesion protein E-cadherin [223]. The Wnt signaling pathway is activated via the binding of ligands to transmembrane receptors encoded by the Frizzled (Fzd) gene family and in conjunction with co-receptors, such as low-density lipoprotein receptors (protein 5 and 6) [237]. This Wnt-Fzd interaction results in dephosphorylation, accompanied by decreased levels of degradation and causes the accumulation of  $\beta$ -catenin in the nucleus [231]. In the absence of Wnt signaling,  $\beta$ -catenin is quickly degraded in the cytoplasm. Without Wnt signaling, phosphorylation of adenomatous polyposis coli (APC) [238] via a cytoplasmic destruction complex results in ubiquitination of  $\beta$ -catenin which is then prone to proteasomal degradation [231]. Additionally, nuclear levels of  $\beta$ -catenin are lessened by their interaction with APC and Axin, both known for their function in transporting  $\beta$ -catenin back to the cytoplasm. In the nucleus, transcriptional corepressors interact with DNA-binding T-cell factor/lymphoid-enhancer factor (Tcf/Lef) proteins, such as Groucho/TLE, and are enabled to block target-gene expression when  $\beta$ -catenin is held at low levels. [239-242]. Wnt binding to the Fzd or low-density lipoprotein receptor protein-membrane receptors results in the accumulation and stabilization of translocated (from cytoplasm to nucleus)  $\beta$ -catenin [237]. Inhibition of such interactions has been noted by secreted Fzd-related proteins, Dickkopfs, and Wnt inhibitory factor-1 (WIF-1) [243, 244].

Since the initial observation that Wnt overexpression results in malignant transformation of mouse mammary tissue [245], aberrant regulation of the Wnt signaling pathway has emerged as a prevalent theme and continues to develop as a fundamental mechanism in broad cancer biology [246]. While Wnt pathway mutations (genetic and epigenetic) are rare in mammary carcinoma, overactive Wnt signaling has been noted in the majority of breast cancers, including rare classes (i.e.: triple -ve type) via several potential mechanisms [145, 233, 247-256]. Several studies to date have indicated that the expression of both Wnt receptors and their ligands are characteristic of breast cancer and furthermore certain receptors and ligands may be breast cancer type specific. In 2004, Bafico *et al.* reported autocrine Wnt signaling in a panel of breast cancer cell lines, including MDA-MB-231, which were identified by the presence of unstabilized  $\beta$ -catenin and then subsequently reduced upon expression or by the addition of the soluble Wnt inhibitors sFRP1 or DKK1 [257]. The expression of the Wnt receptor FZD7 is characteristic of certain rare types of breast cancer [258]. Additionally, the knockdown of FZD7 in cell lines representative of triple -ve breast cancer reduced the expression of Wnt target genes, inhibited tumorigenesis *in vitro* and greatly retarded the capacity of the MDA-MB-231 cell line to form tumors in mice [246, 259]. With respect to Wnt ligands, secreted frizzled related protein (sFRP)-1, an effective competitor and binding site with FZD receptors for Wnt ligands, has been shown to be ectopically expressed in the MDA-MB-231 cell line [260]. This same study showed that the

sFRP1 expressing cells struggled to form tumors upon inoculation into the mammary fat pads of mice and their propensity to metastasize to lung was greatly impaired [260].

Specific to the maintenance of CSCs, Wnt/ $\beta$ -catenin signaling is implicated in many cancers [223, 224, 261-268], including breast cancer [269]. For example, radiation resistance of mouse mammary stem/progenitor cells has been correlated with overexpression of  $\beta$ -catenin in the stem cell survival pathway [266]. Additionally, overexpression of Wnt/ $\beta$ -catenin signaling was reported to promote expansion of the hepatic progenitor cell population in animal studies [267] and the elimination of  $\beta$ -catenin abrogates chemoresistant cell populations endowed with progenitor-like features [57]. Of great interest is the link between Wnt/ $\beta$ -catenin and PI3K (phosphoinositide 3 kinase) /Akt (protein kinase B) pathway as established by several studies. Korkaya *et al.* demonstrated that PI3K/Akt pathway is important in regulating the mammary stem/progenitor cells by promoting  $\beta$ -catenin downstream events through phosphorylation of GSK3 $\beta$  [60, 189]. Other studies have revealed the ability of activated Akt, such as phospho-Akt Ser473 to phosphorylate Ser9 on GSK3 $\beta$ , thereby decreasing the activity of GSK3 $\beta$ , and potentially stabilizing  $\beta$ -catenin [270-272].

In summary, the proof of concept for inhibiting Wnt signaling in cancer is in place. Furthermore there is an increasing amount of evidence to support a role for Wnt signaling in breast cancer; thus, a target has been created for future studies. Specific to breast cancer, the emphasis on target development ranges from antagonizing Wnt ligand secretion or binding to promote  $\beta$ -catenin degradation to specifically blocking  $\beta$ -catenin-mediated transcriptional activity [222]. Nonetheless, as noted several times throughout this chapter, the cooperation of Wnt pathway with other signaling pathways in cancer is an important consideration. Aside from the challenge of determining the most efficacious way to inhibit Wnt related factors, possible safety concerns should be considered; another compelling reason to explore specific targets in the Wnt pathways for all breast cancer sub-types.

### 7.3. Hh pathway

A crucial mediator of normal tissue development, with recent indications as a regulator of tumor-related vascular formation and function [273], the Hh signaling pathway in cancer is activated by ligand independent mutations in the pathway or through Hh overexpression (ligand-dependent) [189, 274, 275]. In the absence of Hh ligands, (Sonic Hh, Desert Hh and Indian Hh), their transmembrane receptor Patched (Ptch) associates with and blocks the G-protein-coupled phosphoprotein receptor Smoothed (Smo) and is only released when secreted Hh ligands bind to Ptch [189, 276, 277]. This binding triggers the dissociation of glioma-associated (Gli) family of zinc finger transcription factors. The three Gli proteins found in vertebrates include Gli1 and Gli2 (thought to activate Hh target genes) and Gli3 (known to act primarily as a repressor) which lead to the transcription of an assortment of genes including cyclin D, cyclin E, myc and elements of EGF pathway effectors through complex interactions with Costal2 (Cos2), Fused (Fu) and Suppressor of Fu (SuFu) [276-278]. Somatic mutations which activate Hh pathway have been implicated in a variety of human malignancies [278] including basal cell carcinomas, pancreatic cancer, medulloblastomas, leukemia, gastrointestinal, lung, ovarian, breast and prostate cancers [274, 275, 279]. Both *in vitro* and mouse model

systems have demonstrated that the Hh signaling pathway plays a crucial role in regulating self-renewal of normal and malignant human mammary stem cells [51, 189]. Hh pathway inhibition has been shown to result in tumor growth inhibition mediated through the stromal microenvironment; as demonstrated in a xenograft model using a tumor and stromal cell co-injection procedure, and consistent with a paracrine signaling mechanism [280]. Although data describing the genetic alteration and the modulation of the expression pattern of Hh pathway components in mammary gland are limited, possible indications for the Hh pathway in development and maintenance of mammary cancer have been proposed [281]. However, a more significant role of Hh signaling has been revealed in prostate cancer studies, demonstrating that autocrine Hh signaling by tumor cells is a requirement for proliferation, viability and invasive behavior [282]. Additionally, the association of accelerated prostate cancer growth and progression with increased Hh signaling has been reported [283]. The Hh signaling pathway has been demonstrated as a critical pathway involved in stem cell self-renewal [276] including the essential role of Hh-Gli signaling in controlling the self-renewal behavior of human glioma CSCs and tumorigenicity [189, 284]. Known for its central role in the control of proliferation and differentiation of both embryonic stem cells and adult stem cells, aberrant activation of Hh signaling could be involved in the generation of CSCs and the development of cancer [278, 285]. In this regard, the development of Hh inhibitors may be a solution in the treatment of human cancers, including prevention of tumor progression. Essential similarities have been noted between Wnt and Hh signaling pathways [286] and their key roles in the physiological and pathological development of both embryonic and stem cells [278] [278] gives rise to the fact that crosstalk exists between the two. Signaling for both are activated by G-protein-coupled receptors [287, 288] and prevents phosphorylation-dependent proteolysis of key effectors (*Cubitus interruptus* or  $\beta$ -catenin) responsible for the conversion of a DNA-binding protein from a repressor to an activator of transcription [278, 289]. Considering the progression model of many cancers, specifically metastasis to bone, it is interesting to note that Wnt signaling has been reported to be downstream of Hh signaling, participating in bone development [278, 290]. Further proof proposing that Wnt signaling is downstream of Hh includes the ability of activated Gli1 to stimulate the transcription of Wnt ligands [276, 278]. It has been noted that molecules involved in Wnt signaling (i.e: GSK-3 $\beta$ ) also play a regulatory role in Hh signaling [278, 286]. Furthermore, canonical Wnt/ $\beta$ -catenin signaling is required for the pathological response to oncogenic Hh signaling [278, 291].

#### 7.4. NF $\kappa$ B Pathway

Along with their hallmark roles in cell survival, proliferation, inflammation and immunity, the NF- $\kappa$ B family of transcription factors are often constitutively expressed in breast cancer tumors [292]. Early studies on NF- $\kappa$ B pathway determined its key role in mammary epithelial proliferation, architecture and branching during early post-natal development [293, 294]. However, independent of its effects on mammary development, evidence exists to suggest that NF- $\kappa$ B regulates breast tumor progression [293, 294]. Constitutive activation of NF- $\kappa$ B in several breast tumor cell lines has been shown to profoundly affect the initiation and progression of breast cancer [295]. NF- $\kappa$ B is also required for the induction and maintenance of the EMT a process that critically controls breast cancer progression [296, 297]. Additionally, it is

evident that NF- $\kappa$ B mostly acts in specific breast cancer sub-types, namely estrogen receptor (ER)-ve and ErbB2+ve tumors [298, 299] and has been implicated in stem cell expansion in breast cancer studies [187].

Activation of NF- $\kappa$ B results in the constant nuclear localization of proteins including p50, p52, p65, cRel and RelB which subsequently up-regulate anti-apoptotic proteins causing an imbalance between normal cell growth and apoptotic cell death [300]. NF- $\kappa$ B-activation occurs mainly through two well characterized pathways, namely the canonical (classical) and the non-canonical (alternative). Both pathways systematically work in a similar fashion in that they are reliant on signal-induced phosphorylation and degradation of an inhibitory molecule to release and transport nuclear NF- $\kappa$ B proteins. However, they differ in the types of trigger signals, activated kinases, inhibitory molecules and NF- $\kappa$ B proteins utilized in each system. In addition to each of these aforementioned pathways, other NF- $\kappa$ B activating pathways exist and have been indicated in the initiation and progression of breast cancer, however we will not discuss these fully in this chapter other than in the context that they appear in the described and relevant research experiments.

Specifically, the canonical pathway involves translocation of a p50/p65 heterodimer to prompt the expression of genes intricated in cell proliferation as well as their survival, inflammatory properties and role in innate immunity [292]. This process occurs through a transforming growth factor beta activated kinase-1 (TAK1)-dependent pathway and is normally dependent on members of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF $\alpha$ ) or IL-1 $\beta$  and other pro-inflammatory cytokines to degrade the inhibitor (I $\kappa$ B $\alpha$ ) by the NF- $\kappa$ B essential modulator ((NEMO)/I $\kappa$ B kinase (IKK)) $\gamma$ -containing IKK complex [292]. In 2004, Biswas *et al.* published results regarding the activation of NF- $\kappa$ B in human breast tumors and in carcinoma cell lines indicating that the canonical pathway contributes to tumor development [298]. The resulting highlight of this experiment gave rise to activated NF- $\kappa$ B as a therapeutic target for distinctive subclasses of ER-ve breast cancers [298]. Specifically, the (NEMO)-binding domain (NBD) peptide (a selective inhibitor of IKK) blocked heregulin-mediated activation of NF- $\kappa$ B and cell proliferation while inducing apoptosis on proliferating cells substantiating the hypothesis that certain breast cancer cells rely on NF- $\kappa$ B for aberrant cell proliferation and simultaneously avoid apoptosis [298]. More recently, Connelly *et al.* [2011] showed, via genetic approaches, that the canonical NF- $\kappa$ B-activating pathway is inhibited in defined frames during polyoma middle T oncogene (PyVT) tumorigenesis and that interruption of this pathway in the mammary epithelium increases the latency of tumors and decreases tumor burden [301].

The non-canonical pathway, considered to be critical in adaptive immunity, is similar to the canonical cascade as it also relies on an IKK $\alpha$  heterodimer, but not on NEMO/IKK $\gamma$  [302]. Prior to nuclear shuttling of 52/RelB dimers, the inhibitory molecule p100 is partially degraded through an NF- $\kappa$ B-inducing kinase (NIK)-dependent pathway [292]. Early studies revealed the enhanced expression of the NF- $\kappa$ B protein p52 in breast cancer samples giving rise to the involvement of the non-canonical pathway [303],[304]. The NF- $\kappa$ B protein RelB is increased in ER $\alpha$ -ve breast cancer cells and is required for the maintenance of mesenchymal ER $\alpha$ -ve breast cancer cells partially through the transcriptional induction of BCL2 [305]. Furthermore, RelB/p52 complexes have since been implicated in mammary carcinogenesis. For example, mouse

mammary tumors induced by 7,12-dimethylbenz(a)anthracene treatment have been shown to increase RelB/p52 activity and the inhibition of RelB in breast cancer cells repressed cyclin D1 and c-Myc levels and growth in soft agar [306]. Perhaps the most conclusive proof of non-canonical NF- $\kappa$ B-activating pathway involvement occurred in studies employing a novel transgenic mouse model to consider the role of involved mediators (downstream of p100/p52) in both mammary development and tumorigenesis [307]. The results of this study indicated an increase in p100/p52 expression in tumors from mice expressing PyVT in the mammary gland, [307] with no change of nuclear p65 detected; an indication that the observation was limited to a deregulated non-canonical NF- $\kappa$ B-activating pathway [307].

#### 7.4.1. NF- $\kappa$ B and breast cancer stem cell renewal

Recently, studies have strengthened the association between stem cells, breast cancer and NF- $\kappa$ B. Such have been captured in a review by Shostak and Chariot highlighting experiments to date complemented by a compelling rationale for targeting NF- $\kappa$ B and other developmental pathways involved in the self-renewal of normal stem cells [187]. The involvement of NF- $\kappa$ B in various signaling cascades has proven critical in several studies involving breast cancer stem cell expansion.

Cao *et al.* [2007] showed that IKK $\alpha$  is both a regulator of mammary epithelial proliferation and a contributor to ErbB2-induced oncogenesis [308]. Specifically, breast cancer cells from IKK $\alpha$  (AA/AA) knock-in mice (whereby IKK $\alpha$  activation is disrupted) crossed with the Her2 murine breast cancer model, exhibited diminished self-renewal capacity and resulted in the inability to establish secondary tumors [308]. Breast cancers that generate primary, as opposed to secondary mammospheres such as seen in mice used in these experiments, suggests that IKK $\alpha$  is likewise required for the self-renewal of tumor-initiating cells from the Her2 breast cancer model [187]. Additionally, mutated IKK $\alpha$  slowed tumor development following exposure to 7,12-dimethylbenzaanthracene or the MMTV-c-neu (ErbB2/Her2) transgene; however there was no effect on MMTV-v-Ha-ras-induced cancer despite the fact that both of these oncogenes rely on cyclin D1 [308]. In this same series of studies, carcinoma cells from another mouse model (IKK $\alpha$ (AA/AA)/MMTV-c-neu) underwent premature senescence when cultured under conditions used for propagation of mammary gland stem cells. Altogether, these mouse models of breast cancer show that IKK $\alpha$  seems to act as a central protein in the activation of NF- $\kappa$ B during breast cancer stem cell self-renewal [308]. Therefore, the researchers concluded that IKK $\alpha$  may represent a novel and specific target for treatment of ErbB2+ve breast cancer.

While NF- $\kappa$ B appears to be activated in luminal progenitor cells during differentiation of mammary colony-forming cells [309], the mammary stem-like basally located cells are devoid of NF- $\kappa$ B activity [309, 310]. Taken together, these studies suggest that only the canonical NF- $\kappa$ B pathway is active in normal luminal progenitor cells before transformation and is required for the formation of mammary luminal-type epithelial neoplasias [309]; a reminder of the importance of understanding the cellular etiology underpinning breast tumor heterogeneity [310].

Another interesting role for NF- $\kappa$ B signaling involves the link between inflammation and cancer. While the mechanism linking inflammation and cancer has yet to be explained, we do know that the inflammatory cytokine, interleukin(IL)-6 is up-regulated in epithelial cancers, including breast cancer [311]. We also know that NF- $\kappa$ B regulates the expression of anti-apoptotic genes and activates different pro-inflammatory cytokines and chemokines, including IL-6 [312, 313]. Further clarity on the interactions of NF- $\kappa$ B signaling, inflammation and cancer has been gained through a study showing that the temporary activation of Src oncoprotein mediates an epigenetic event whereby immortalized breast cells are stably transformed to a cell lines that represent self-renewing mammospheres containing cancer stem cells [313]. The inflammatory response triggered by the activation of Src and further downstream signaling which inhibits IL-6 expression is mediated by NF- $\kappa$ B [313]. It has been shown that the transformation of cells utilized within this experiment occurs via a positive feedback loop whereby IL-6 mediated STAT3 transcription factor stimulation activates NF- $\kappa$ B [313]. These authors have demonstrated that Src activation triggers a rapid inflammatory response mediated by NF- $\kappa$ B that is critical for cellular transformation. While this study defines Src's role as an oncogenic kinase promoting the expansion of breast cancer stem cells, it also demonstrated the critical involvement of NF- $\kappa$ B in the process [313].

It is known that the onset of progestin-driven breast cancer is affected by the deletion of IKK $\alpha$  in mammary-gland epithelial cells [314]. Such studies are relevant in breast cancers as they consider the importance of associated risk factors between hormone replacement therapy (i.e.: progesterones or synthetic derivatives) and the increased risk of incident of fatal breast cancer [314]. The expression of both receptor activator of NF- $\kappa$ B (RANK) and RANK ligand (RANKL) have been observed in primary breast cancers in humans and breast cancer cell lines [315]. Studies to date indicate that the RANKL/ RANK system is mediated in part by IKK- $\alpha$ -NF- $\kappa$ B signaling and controls the incidence and onset of progestin-driven breast cancer; more specifically a loss of RANK expression significantly impairs the self-renewal capacity of cancer stem cells [314, 316]. Thus, because of the link between the RANKL/RANK system and progestin-driven epithelial carcinogenesis, RANKL inhibition could be considered as a novel approach to the prevention and/or treatment of breast cancer [314].

More recently, a model of Her2-dependent tumorigenesis indicated that breast cancer stem cell renewal is regulated by epithelial NF- $\kappa$ B through a reduction in the expression of key embryonic stem cell regulators, namely Sox2 and Nanog [317]. Specifically, NF- $\kappa$ B was required for both proliferation and colony formation of Her2-derived murine mammary tumor cell lines [317]. Additionally, the rate of initiation of Her2 tumors was governed by NF- $\kappa$ B [317].

### 7.5. Summarizing pathway interruptions/targets

In many human breast cancers, all three developmental pathways (Wnt, Notch and Hh) appear to be deregulated and control the self-renewal of normal stem cells from a molecular perspective [186]. Additionally, the involvement of NF- $\kappa$ B has emerged as another involved pathway in breast cancer based on what we know about Her2, a membrane bound receptor tyrosine kinase. Her 2 is overexpressed in 30% of breast cancers and critically controls the cancer stem-cell population [318]. Since Her2 activates NF- $\kappa$ B through the canonical pathway [319] [27],

the hypothesis exists that the NF- $\kappa$ B pathway may be involved in the biology of breast cancer stem cells. It is also obvious that these interrupted pathways, and likely others unknown at this time, are responsible for certain stages of cancer progression or cancer cell aggression. If so, then we are in agreement with others who have noted that the development and identification of selective inhibitors of specific signaling pathways is an attractive approach for the prevention of tumor progression and/or treatment of cancers [278]. However, we have also mentioned several examples of cross-talk between the components of different cell signaling pathways. This concept then introduces the task of targeting multiple pathways in an effort to prevent progression and metastasis of cancers. Alternatively, and based on the premise that certain pathways operate downstream of others, it would be more reasonable to focus on inhibitors of overarching pathways, such as NF- $\kappa$ B. In summary, such oncogenic pathway signatures are fundamental in natural product testing. Therapeutic approaches involving natural products may provide a link between pathway deregulation and therapeutic sensitivity indicating an opportunity for the development of target compound(s).

## **8. Prevention and treatment of breast cancer with natural products: Past successes and promising future treatments**

Currently, hormones and cytotoxic drugs remain the standard treatment for metastatic breast cancers. Development of a therapeutic approach for treating tumors, tumor reoccurrence and metastatic tumors is crucial for reducing mortality in cancer patients [6]. There is an urgent need to explore agents that will be effective in preventing and treating metastasis of breast cancer. For centuries, nature has provided us with a rich source of compounds for various disease treatments. Such naturally-derived molecules have been utilized in formal drug discovery platforms of the pharmaceutical industry. Greater than 60% of new chemical entries at the National Cancer Institute from 1981-2002 were either natural products or were derived from natural products [320]. Such naturally-occurring sources can be defined by their origin and include biotic (i.e.: forests, plants, animals, birds and marine organisms) and abiotic (i.e.: land, water, air and minerals such as gold, iron, copper, and silver) components [321]. Within these categories, plants have proven to be a rich source of lead compounds (i.e: alkaloids, morphine, cocaine) or the basis for synthetic drugs (i.e: anesthetics from cocaine) [320, 321]. The complexity and variation of plant structures indicates that their evolution has naturally completed the screening process and that the creation of potent compounds makes them more likely to survive. Plants offer the advantage of abundance, and even with such clinical successes as paclitaxel (Taxol) from the yew tree, and the antimalarial agent artemisinin from *Artemisia annua*, the vast majority have not been studied [320]. Due to promising bioactivity and diversity, the plant environment offers a potential source of natural products. A vast number of studies in the discipline of epidemiology have confirmed an association between fruit and vegetable consumption and the reduced risk of several cancers resulting in an increased interest in the role of naturally occurring dietary compounds in the efficacy of cancer chemoprevention [322]. Thus, the exploration of plant-based molecules as anti-cancer drugs is appropriate.



The history of Tamoxifen and its derivatives in the successful treatment of estrogen receptor (ER)+ve breast cancers are well documented. In the past Tamoxifen was successful in reducing breast cancer mortality rate in hormone receptor+ve breast cancer patients by up to a third and thus was the stronghold of endocrine treatment [323]. Clinical trials have indicated that aromatase inhibitors (AI) have improved efficacy compared with Tamoxifen for the treatment of post-menopausal hormone receptor+ve patients [324-327]. Additionally, the response rate for third generation AIs as first-line agents range from 30%–50% in ER+ve advanced breast cancer. [323]. Leading to these discoveries, and in an effort to capitalize on the advantages of both anti-aromatase and anti-estrogenic activity, many natural products have been tested for their ability to prevent and treat breast cancer, *in vivo* and *in vitro*. Table 4 summarizes plant based compounds that have been indicated for their potential as a prevention or treatment in breast cancer. This list is not exhaustive; however it does capture the extracts studied to date according to activity in cell lines and animal models and represents the most common types of breast cancer. In addition to those listed within Table 4, soy-based extracts, curcumin and piperine have been studied and we will discuss these in detail as the most promising plant based targets in the prevention, treatment and progression of breast cancer.

## 9. Promising plant based targets in the prevention, treatment and progression of breast cancer

### 9.1. Summarizing the individual and combined effects of the soy isoflavones, genistein and daidzein, on mammary tumor development, metastases and invasive breast cancer cells *in vivo* and *in vitro*

Genistein, daidzein and glycitein are the main isoflavones present in soybean and soy-based foods [341] [342]. Out of these, genistein is the mostly studied and dominant isoflavone of soy against breast cancer and has progressed to phase II clinical trials [343]. Soy isoflavones acting upon breast cancer cells *in vitro* and *in vivo* have been studied extensively with varying results and the clinical implications specific to breast cancer have been discussed. Soy isoflavones are structurally similar to female androgen estrogen, and thus they are also known as phytoestrogens [344] and may possibly be competing with the physiological estrogens. Genistein and daidazine (but not glycitein) possesses the ability to transactivate the estrogen receptors.

Utilizing cell based assays on MCF-7 human breast cancer cells, estrogenic agonist actions of soy isoflavones have been studied by Matsumura and co-workers whereby genistein and daidzein exert estrogen response in MCF-7 cells [345] via ER with higher affinity to ER $\beta$ 1. Similarly, in hepatoma cells transfected with ER, genistein and daidazine bind to both ER $\alpha$  and ER $\beta$  but with more affinity to ER $\beta$ . Genistein is more potent compared to daidazine [346]. However, these phytoestrogens are 400-600 times less potent compared to 17- $\beta$  estradiol [347].

*In vitro*, genestein is capable of identifying cells that specifically carry BRCA1 mutation and strongly inhibits the growth of BRCA1 mutant cells compared to cells expressing the wild-type BRCA1 protein [348]. The resistance shown by cells expressing wild type BRCA1 protein has been attributed to increased AKT and decreased p21 (WFA1/CIP1) protein levels [349].

Natural Product; Active Ingredient	Effect	Ref.
White button mushrooms ( <i>Agaricus bisporus</i> ); Conjugated linoleic acid and its derivatives	Decreased both tumor cell proliferation and tumor weight with no effect on rate of apoptosis in MCF-7aro cells and nude mice injected with MCF-7aro cells.	[324]
<i>Taxus brevifolia</i> (Pacific Yew) and other <i>Taxus</i> derivatives; Paclitaxel	Promotes tubulin polymerization and stabilization of microtubules against depolymerization.	[328] [329]
Dysoxylum binectariferum; Flavopiridol	Inhibition of MMP-2 and MMP-9 secretion of in MDA-MB-435 (parental) and 435.eB (stable transfectants) breast cancer cells.	[330]
Green Tea; <i>Epigallocatechin gallate</i>	Suppresses receptor (ER $\alpha$ ) MBA-MB-231 breast cancer cell growth <i>in vitro</i> and <i>in vivo</i> in combination with curcumin.	[331]
Bloodroot ( <i>Sanguinaria Canadensis</i> ); Sanguinarine	Induced apoptosis through mediation of ROS production in MDA-MB-231 breast carcinoma cells, decrease in mitochondrial membrane potential, release of cytochrome c, activation of casp-3, and casp-9 and down regulation of Bcl-2.	[332]
<i>Garcinia hanburyi</i> . (Gamboge tree); Gambogic acid	Upregulation of p53 and down regulation of Bcl-2 resulting in apoptosis in MCF-7 cancer cells.	[333]
<i>Ganoderma lucidum</i> ; Ganoderic acids	Inhibits AP-1 and NF- $\kappa$ B activity; inhibition of u-PA secretion from MDA-MB-231 cells.	[334]
Ginger; Acetoxychavicolacetate	Decreased cell viability in MCF-7 and MDA-MB-231 cells via casp-3-dependent increase in apoptosis.	[335]
Grapes, fruits, and root extracts of the weed <i>Polygonum cuspidatum</i> ; Resveratrol	Suppresses NF- $\kappa$ B activation and cell proliferation in MCF-7 cells, reduced expression of Cox-2 and MMP-9 (with a reduced NF- $\kappa$ B activation).	[336]
<i>Garcinia indica</i> (kokum); Garcinol	Induced apoptosis in MCF-7 and MDA-MB-231 cells via caspase activation and down-regulation of NF- $\kappa$ B regulated genes.	[337]
<i>Plumbago europaea</i> ( <i>Plumbago</i> ); Plumbagin	Induced apoptosis with concomitant inactivation of Bcl-2 and the DNA binding activity of NF- $\kappa$ B in MCF-7aro breast cancer cells.	[338]
Rotenone; Deguelin	Arrests cells at the S phase resulting in anti-proliferative effect in MDA-MB-231 cancer cells.	[339]
Silymarin; Silibinin	Reduced PMA-induced invasion of MCF-7 cells through specific inhibition of AP-1-dependent MMP-9 expression.	[340]

**Table 4.** Plant Based Natural Products indicated in Breast Cancer

Cyclooxygenase-2 (COX-2) expression, which is associated breast cancer risk [350], can also be inhibited by soy isoflavones [351]. Thus, it seems that soy isoflavones are capable of curtailing breast cancer risk factors.

The influences of soy isoflavones on cell growth, cell cycle and apoptosis are all relevant to their effectiveness as chemopreventive agents for breast cancer. A number of studies have indicated the potential of genistein to inhibit proliferation of breast cancer cells in culture by causing cell cycle arrest and/or apoptosis. Genistein induces G2/M cell cycle arrest [352-354]. This effect was seen both in hormone sensitive and hormone independent cells [352]. According to Li *et al.* [2008] G2/M cell cycle arrest occurs, via stable activation of ERK1/2 pathway [354].

Demarcation on the relative importance of cell adhesion, invasion and migration for primary tumor growth verses metastatic tumour growth is not clear. However, motility, migration and adhesion are more connected to metastasis which is undoubtedly the most life-threatening aspect of breast cancer. Thus, it is crucial to identify the effects of soy isoflavones on disease metastasis.

Microarray analysis of genistein treated HCC1395 cells, a cell line derived from an early stage primary breast cancer, has indicated up-regulation of genes that inhibit invasion and down-regulation of genes that promote invasion [355]. Genistein enhances the adhesion of breast cancer cells [356, 357]. This may possibly be one method utilized by genistein to reduce metastasis.

A study by Vantighem *et al* (2005) describes the ability of dietary genistein to affect metastasis in a post-surgical model in mice [358]. This test model mimics the clinical situation where primary tumors are surgically removed and therapeutic strategies are applied to prevent the growth of any cancer cells seeded to other locations prior to surgery. In this study, primary tumours were established by injecting human breast carcinoma cells, MDA-MB-435/HAL, into the mammary fat pad of nude mice. After 5 weeks, tumours were surgically removed and mice were maintained with a soy free diet or genistein supplemented diet. At the end of 5 weeks, a 10 fold reduction in percent lung metastasis in mice fed on a genistein supplemented diet was seen. In another study, as described by Zhang *et al.*, genistein has shown its ability to reduce the number and volume of osteolytic bone metastases in Balb/c(nu/nu) mice injected with MDA-MB-231 human breast cancer cells [359]. As there are clear indications of the ability of this compound to inhibit breast cancer metastasis, as described in the various animal studies, it would be useful to identify the associated molecular mechanisms. Although there are no conclusive findings, a number of different mechanisms have been suggested.

In studies focused on determining related mechanisms, scientists have given more attention to molecules that are overtly expressed in malignant breast tumours. For example, much attention has been invested into the actions of focal adhesion kinase (FAK), a tyrosine protein kinase. As described previously, cell motility is an integral part of metastasis and it is justifiable to investigate the components directly involve in cell motility. FAK has been designated as a regulator of cell migration and invasion [360]. Since over expression of FAK in human tumors occurs, it has been proposed as a potential therapeutic target [361]. Increased expression of FAK expression in invasive breast carcinomas is associated with an aggressive phenotype [362]. In a transgenic model of breast cancer, mammary epithelial specific disruption of FAK blocks transition of premalignant hyperplasias to carcinomas (and their subsequent metasta-

sis) indicating direct involvement in mammary tumor progression [363]. Further, attenuation of FAK function dramatically increased apoptosis in breast cancer cells [364]. Disruption of FAK signaling by expressing the N-terminal domain FAK in human breast carcinoma cells has led to rounding, detachment and apoptosis [365]. To gain insight into the influence of genistein and daidazine in this important pathway in breast cancer, *in vitro* and *in vivo* studies have been conducted. According to an *in vitro* study, the soy isoflavones genistein, daidzein and 17 $\beta$  estradiol increased the number of focal adhesions and FAK activity in ER $\alpha$  +ve (T47D cells) as well as in ER $\alpha$  -ve (MDA-MB-231) breast cancer cells indicating possible involvement of novel signaling pathways and independent of estrogen receptors. Authors of this study suggested a progressive role (to metastasis) for soy isoflavones in the activation of multiple FAK regulated signaling pathways relevant to breast cancer [366], however the mechanism was not investigated. The studies of Mitra and co-workers may possibly explain the mechanism of FAK in breast cancer metastasis [367]. According to this study, reduced FAK activity or expression blocked 4T1 breast cancer cell invasion through matrigel and the blocking was associated with a 2-3 fold reduction in the expression of urokinase plasminogen activator (uPA) [367]. uPA is a serine protease that cleaves extracellular matrix and stimulate plasminogen to plasmin. Cancer cells are known to digest the ECM via substances like uPA and matrix metalloproteinases (MMPs) as a means of invading surrounding tissue. This idea is supported by the fact that breast cancer patients with higher level of MMP-9 in tissue is associated with lymph node metastasis; thus, MMP-9 levels in serum, tumour tissue and urine are used as prognostic markers [368]. Furthermore, a study on the role of membrane-type 1 matrix metalloproteinase (MT1-MMP) *in vitro* and in SCID mice reports that the down regulation of mammary cancer cell MT1-MMP has no effect on primary tumour growth and lymph node metastasis, but reduces the occurrence of lung metastasis [369]. Interestingly, uPA secretion from mammary carcinoma cells can be influenced by genistein. This property of genistein has been shown *in vitro* and *in vivo* and the implication on tumour angiogenesis has been studied using F3II mammary carcinoma cells in culture as well as in a syngeneic mouse model. Accordingly, non-cytotoxic concentrations of genistein (0.1-50 $\mu$ M) significantly reduced motility in F3II mammary carcinoma cells and inhibited the secretion of uPA from cell monolayers. Once F3II cells were implanted in syngeneic mice receiving a treatment of genistein (10mg/kg/day), anti angiogenic effects were evident [357].

These studies indicate the effectiveness of genistein to inhibit angiogenesis and metastasis by inhibiting proteolytic substances such as uPA. In this respect, our attempt to further explore the value of soy isoflavones in modulating metastasis enabled us to review some important findings. Studies by Shao *et al*, [1998] reported that genistein inhibited the invasion of MCF-7 and MDA-MB-231 cells *in vitro* and the inhibition was characterized by down regulation of MMP-9 (matrix metalloproteinase-9) and up regulation of TIMP-1 (tissue inhibitor of metalloproteinase-1) [370]. The same effects were seen in nude mouse xenografts of MCF-7 and MDA-MB-231 cells [371]. Furthermore, in MDA-MB-231 xenografts, genistein inhibited tumour growth, stimulated apoptosis, regulated p21 WAF1/CIP1 expression, inhibited angiogenesis with reduced vessel density and decreased the levels of vascular endothelial growth factor and transforming growth factor  $\beta$ 1 [370]. Further studies by the same authors reported that genistein inhibits both constitutive and epidermal growth factor stimulated

invasion in ER- human breast carcinoma cells as characterized by up regulation of TIMP-1 as well as other trypsin inhibitors like protease nexin-II (PN-II) and alpha 1-antitrypsin (alpha 1-AT) [371]. Kousidou *et al* [2005], examining normal mammary cells (MCF-12A), low invasive (ER+ve) MCF-7 cells and high invasive MDA-MB-231 (ER-ve) cells (in parallel) showed differences in the effect of genistein on highly invasive and low invasive cells. Accordingly, all cell types expressed genes of MMP-2, MMP-9, membrane-type matrix metalloproteinase (MT-1, MT-2, MT-3), MMP and TIMP-1, -2 and -3. However, once genistein was added, down regulation of all MMP genes in highly invasive cells and down regulation of many genes in low invasive MCF-7 cells was observed [372].

Based on the above findings, genistein has a role in reducing metastasis and this appears to arise from its ability to suppress uPA and MMPs thereby invading barriers with no direct effect on the capacity of cell mobility. According to the literature, expression of uPA and MMPs is regulated by NF- $\kappa$ B [373] [307]. Therefore it would be worthwhile to review any association between genistein and the NF- $\kappa$ B pathway.

#### 9.1.1. Genistein may act via inhibition of the NF- $\kappa$ B pathway

The possible connection between NF- $\kappa$ B and breast cancer has been extensively studied [374]. Using a doxycycline-inducible new mouse model to inhibit NF- $\kappa$ B activity, specifically within the mammary epithelium at the time of tumor development, Connelly *et al* (2011) indicated the active contribution of NF- $\kappa$ B in mammary tumor progression [301]. In this model, inhibition of NF- $\kappa$ B activity showed an increase in tumor latency and a decrease in tumor burden [301]. Specifically, soy isoflavones inhibited the tumors by suppressing the NF- $\kappa$ B pathway. Furthermore genistein potentiates the activity of a number of NF- $\kappa$ B mediated chemotherapeutic agents by increasing apoptosis in various cancer cells, including MDA-MB-231 breast cancer cells [375]. In the same cell line, genistein induces G2/M cell cycle arrest via stable activation of ERK1/2 pathway [354],[376]. Furthermore, the MDA-MB-231 cell line has the ability to selectively block NF- $\kappa$ B transactivation of IL-6, a cytokine that is known for estrogen independent tumorigenesis activity [377]. Inhibition of proteasome activity by genistein in MCF-7 breast cancer cells has also been associated with NF- $\kappa$ B inhibition [378]. This property of genistein is particularly important for ER deficient breast cancer as constitutive NF- $\kappa$ B and Mitogen- and Stress- Activated Protein Kinase-1 (MAPK) /MSK activity are linked with aggressiveness and the metastasis.

It is clear that almost all of the studies that show beneficial effects of soy isoflavones utilized genistein. However, within the natural products of soy and soy food not only genistein, but daidzein and glycitein are present. A recent study testing genistein, daidzine and glycitein separately has indicated interesting results. According to this study, only genistein induced apoptosis in MCF-7 breast carcinoma cells whereas daidzein caused a slight cell-stimulating effect in the absence of E2; thus, the authors pointed toward the possible risk of breast cancer in postmenopausal women who take soy supplements [379]. This statement is important as a number of soy supplements available in the market contain high levels of daidzein [380, 381].

An animal study using nude mice and MDA-MB-435 breast cancer cells reported the individual and combined soy isoflavones exerting differential effects on metastatic cancer progression

[382]. As described in this study, daidazine increased mammary tumour growth by 38% while genistein decrease tumor growth by 33%. Moreover, the combined isoflavones increased metastasis to all the organs examined, although no effect on primary tumour growth was noted. These results have led authors to include the consumption of soy foods as a cause of increased breast cancer metastasis. Also, a number of studies by Ju *et al* [383-385] have cited enhanced growth effects of soy components in ER+ve breast cancers. However, with these studies conducted in immune compromised mice, the relevance of these findings have been criticized [386], especially as pre-treatment with genistein has shown to be protective against mammary tumors [387]. More recently, the antiproliferative activity of both genistein and quercetin has been indicated in the prevention and treatment of HER2-overexpressing breast cancer via inhibition of NF $\kappa$ B signaling [388]. In this study, these specific phytoestrogens inhibited proliferation in MCF-7 cell lines accompanied by an increase in intrinsic apoptotic indicators, induction of the extrinsic apoptosis pathway (up-regulating p53), a reduction in the phosphorylation level of I $\kappa$ B $\alpha$ , and negated the nuclear translocation and subsequent phosphorylation of nuclear p65 [388].

#### 9.1.2. Sources of soy

Soybeans can be considered as the richest source of isoflavones in the human diet [389, 390], and are available in fermented and non-fermented forms. Fresh green soy beans, whole dry soybeans, whole-fat soy flour, soy milk and soymilk products such as tofu, okara and yuba are non-fermented while soy sauce, tempeh, miso and natto are fermented products [391]. Additionally, products such as soy dairy substitutes, soy cheese, soy yogurt, and soy burgers seem to be popular in Western countries. The isoflavone content in various soy-based food products greatly differ. Other than soy food products, soy supplemented (categorized as a class of complementary medicine) nutraceuticals are widely consumed by Western communities. The quality and standard of these supplements are questionable. For instance, a survey carried out in the Eastern Washington Region of U.S.A. tested 13 products (7 tablet and 6 capsule formulations) by HPLC and showed that only 4 of the 13 products contained the minimum of 90% isoflavone content claimed on the label and variations in composition over time were noted [392]. Interestingly, a recent review shows that overall, most commercially available nutraceuticals are poor in quality [393].

#### 9.1.3. Clinical studies

Although there have been a large number of studies carried out to evaluate a possible soy-breast cancer link, evidence is inconclusive. The two main theories tested involve the effect of soy isoflavone consumption in risk of breast cancer incidence and its effect on recurrence. The largest population based cohort study, including 5,042 female breast cancer survivors, shows that soy food consumption is significantly associated with decreased risk of death and recurrence [394]. Another cohort of 1,954 female breast cancer survivors, who consumed soy isoflavones at the levels comparable to the Asian population, while undergoing tamoxifen therapy showed a reduction in the risk of cancer recurrence and no interference in the efficacy of tamoxifen [395]. Further studies have indicated that soy intake prior to cancer diagnosis is

unrelated to disease-free breast cancer survival and that the association between soy protein intake and breast cancer survival does not differ according to the presence of other risk factors such as ER/PR status, tumor stage, age at diagnosis, body mass index (BMI), waist to hip ratio (WHR), or stage of menopause [396]. No variations were noted in the soy-survival association of indicated polymorphisms in ER $\alpha$  and ER $\beta$  indicating that soyfoods do not have an adverse effect on breast cancer survival. A recent meta-analysis by Dong and Qin (4 studies of breast cancer recurrence and 14 studies of breast cancer incidence) revealed that the consumption of soy isoflavone is inversely associated with risk of breast cancer incidence [397]. However, the protective effect is only observed among studies conducted in Asian populations, unlike those reported in Western populations [397]. One of the previous meta-analysis studies by Wu *et al* [2008] show a similar trend. Accordingly, in Asian populations a higher intake of soy isoflavones, as compared with lower intake, is associated with 29% reduction in the risk of developing breast cancer [398]. Hence, the consumption of soy food at levels similar to those consumed by Asian populations may have protective effects. However, there is evidence in the literature to show possible adverse effects of soy due to its known stimulatory effect on the premenopausal female breast as indicated by increased secretion of breast fluid, the appearance of hyperplastic epithelial cells and elevated levels of plasma estradiol [399]. Some animal studies support the idea of related disadvantages of consuming soy isoflavones. In ovariectomized athymic nude mice, physiological concentrations of dietary genistein stimulates the growth of estrogen dependent MCF-7 tumors in a dose dependent manner [383, 400]. Furthermore, the same test model showed that dietary genistein reverses the inhibitory effect of tamoxifen on the growth of MCF-7 tumors [384].

## **9.2. The role of curcumin and piperine in breast cancer prevention and its effects on normal human breast stem cell renewal and signaling**

Curcumin is a plant derived polyphenol which gives rise to the yellow colour in the spice, tumeric. This pigment is obtained from the plant *Curcuma longa* and has been noted to have power against cancer. In their review of the mechanisms of cell cycle regulation by curcumin, Gaurisankar and Das have named it as a multiple edged sword [401] because of its ability to regulate the cell cycle as well as apoptosis. Distorted cell cycle regulation and programmed cell death/apoptosis are characteristic features of cancer and curcumin has been shown to target both mechanisms. The ability of curcumin to inhibit telomerase activity [402] and to disrupt mitotic spindle structure causing [403] micronucleation in MCF-7 breast carcinoma cells has been reported. Also, curcumin is known to induce anti-proliferative activity via the decreased expression of cyclin D1 and CDK-4 in MCF-7 breast carcinoma cells [404] and can induce apoptosis through p53 dependent Bax induction [405]. Curcumin is able to disrupt breast tumor growth, but also to inhibit metastasis.

As with genestein, curcumin has been shown to mediate its anti-cancer effects via regulation of the NF- $\kappa$ B signaling pathway. In the nude mouse model, curcumin suppresses the paclitaxel-induced NF- $\kappa$ B pathway resulting in the inhibition of lung metastasis of human breast cancer [406]. The modification of NF- $\kappa$ B signaling eventually leads to pro-apoptotic events and perhaps inhibition of ECM breakdown. Curcumin induced apoptosis in MDA-MB-231 cells

*in vitro* is associated with I $\kappa$ B and p65 phosphorylation and hence reduced activation of NF $\kappa$ B [407]. This leads to reduced expression on MMPs, diminished invasion through a reconstituted basement membrane and a lower number of metastases in immunodeficient mice injected with tumor cells via intra cardiac route [407]. The high level of of MMP-3 expression noted in MDA-MB-231 invasive breast carcinoma cells is not evident on MCF-7 non-invasive breast cancer cells, implicating its importance in invasion and metastasis. The possibility of using the major forms of curcuminoids, curcumin, demethoxycurcumin, and bisdemethoxycurcumin (all of which are found in turmeric powder) as MMP-3 inhibitors to modulate MMP-3 expression has been suggested [408]. According to Chiu and Su (2009), curcumin inhibits proliferation by increasing the Bax to Bcl-2 ratio while inhibiting the migration via decreasing NF- $\kappa$ B p65 expression in breast cancer MDA-MB-231 cells [409]. Utilizing microarray gene expression analysis on MDA-MB-231 breast cancer cells, Bachmeier *et al.* demonstrated the ability of curcumin to downregulate inflammatory cytokines CXCL-1 and -2 via suppression of NF- $\kappa$ B translocation. Moreover, silencing CXCL-1 and -2 resulted in a downregulation of several metastasis promoting genes [410].

Interestingly, curcumin can interfere with estrogen-mimicking pesticides such as endosulfane, DDT and chlordane [411].

### 9.2.1. Sources of curcumin

Curcumin is generally considered to be the most active component and the principal curcuminoid found in tumeric [411]. The spice tumeric is commonly used in curries and contains 2-8% of this active ingredient [412], however a supplement form is also available.

### 9.2.2. Clinical trials

Little data is available on the pharmacokinetics and metabolism of curcumin in humans. Dose-limiting toxicity is not reported and high oral doses of curcumin (up to 12g/day) have been tested [220, 413, 414]. In a phase one clinical trial involving individuals with non-invasive cancer and pre-cancerous conditions, oral dosing of 4g, 6g and 8g of curcumin yielded peak serum concentrations of 0.51 +/- 0.11microM, 0.63 +/- 0.06 microM, and 1.77 +/- 1.87 microM, respectively. Peak serum concentrations of curcumin are seen 1-2 hours after oral intake and this gradually declines within 12 hours [220]. In another phase I clinical trial involving 15 patients with advanced colorectal cancer, 3.6g of curcumin daily for up to 4 months was well-tolerated [413]. Another study examined the pharmacokinetics of 450mg-3600mg curcumin (daily for 1 week) in twelve patients with hepatic metastatic disease from primary colorectal adenocarcinomas. Using a high-performance liquid chromatography assay, low nanomolar levels of the parent compound and its glucuronide and sulphate conjugates were found in the peripheral or portal circulation; despite its absence in liver tissue, trace levels of products of its metabolites were detected [415]

Due to the poor bioavailability of curcumin systemically, high priority has been given to study its potential against colorectal cancers. A very recent publication on a phase IIa clinical trial involving men and women 40 years of age or over and smokers that carry 8 or more colorectal



aberrant crypt foci (ACF) indicates that oral dosing of curcumin (4g per day for 30 days) significantly reduces colorectal ACF, a biomarker of colon carcinogenesis. [416] The reported anti-carcinogenic effect of curcumin is not associated with increased levels of curcumin in local tissue but increased levels of conjugate concentrations in suggesting that curcumin may mediate its effects by curcumin conjugates delivered systemically. The same study showed that the presence of curcumin conjugates in plasma and tissue prior to treatment (believed to be originated from the normal diet of the studied population) were accompanied by a steady increase of curcumin conjugates following the month-long daily dosing [416]. A study examining the pharmacokinetics of curcumin at the concentrations of 10g and 12g in twelve healthy volunteers indicates comparable results. Accordingly, a single dose of orally administered curcumin resulted in the detection of conjugates, glucuronides and sulfates in plasma in all subjects while free curcumin was evident in only one subject [414]. Even though curcumin conjugates and other breakdown products have not been assessed for their anticarcinogenic properties [416], these findings shed some light on the potential of curcumin as a treatment of all cancers, including those of colorectal origin. This may offer a likely explanation of how continuous exposure to small quantities of curcumin via normal diets protects Asian women from breast cancer.

A phase I dose escalation trial of combined effects of docetaxel and curcumin in patients with advanced and metastatic breast cancer was published very recently. This study involved 14 patients and demonstrated the feasibility, safety and tolerability of a combination of curcumin with a standard dose of docetaxel which warrants further investigation and progression to a Phase II clinical trial [417]. Similarly, the curcumin inhibiting effects of chemotherapy induced apoptosis in models of human breast cancer have been identified [418].

### 9.2.3. Curcumins' ability to destroy cancer stem cells

The properties of CSC's are connected with major signaling pathways. The signaling pathways active in mammary stem cells are shown to be Wnt/ $\beta$  catenin, Hh and Notch [60],[51],[185],[206],[269].

A recent study by Karkarala *et al.* has demonstrated the potent inhibitory effect of curcumin and piperine on Wnt/ $\beta$ -catenin signaling in primary human breast epithelial cells [419]. In this study, inhibition of Wnt signaling pathway was shown to affect breast stem cell renewal by inhibiting the mammosphere formation. According to the authors, curcumin and piperine (separately and in combination) inhibited breast stem cell self-renewal; however toxicity to differentiated cells was not reported. The plasma concentration of curcumin in people taking high oral doses has been shown to be very low due to many reasons such as metabolism of the compound in the intestine and the liver, as reviewed by Burgos-Moron *et al.* [420].

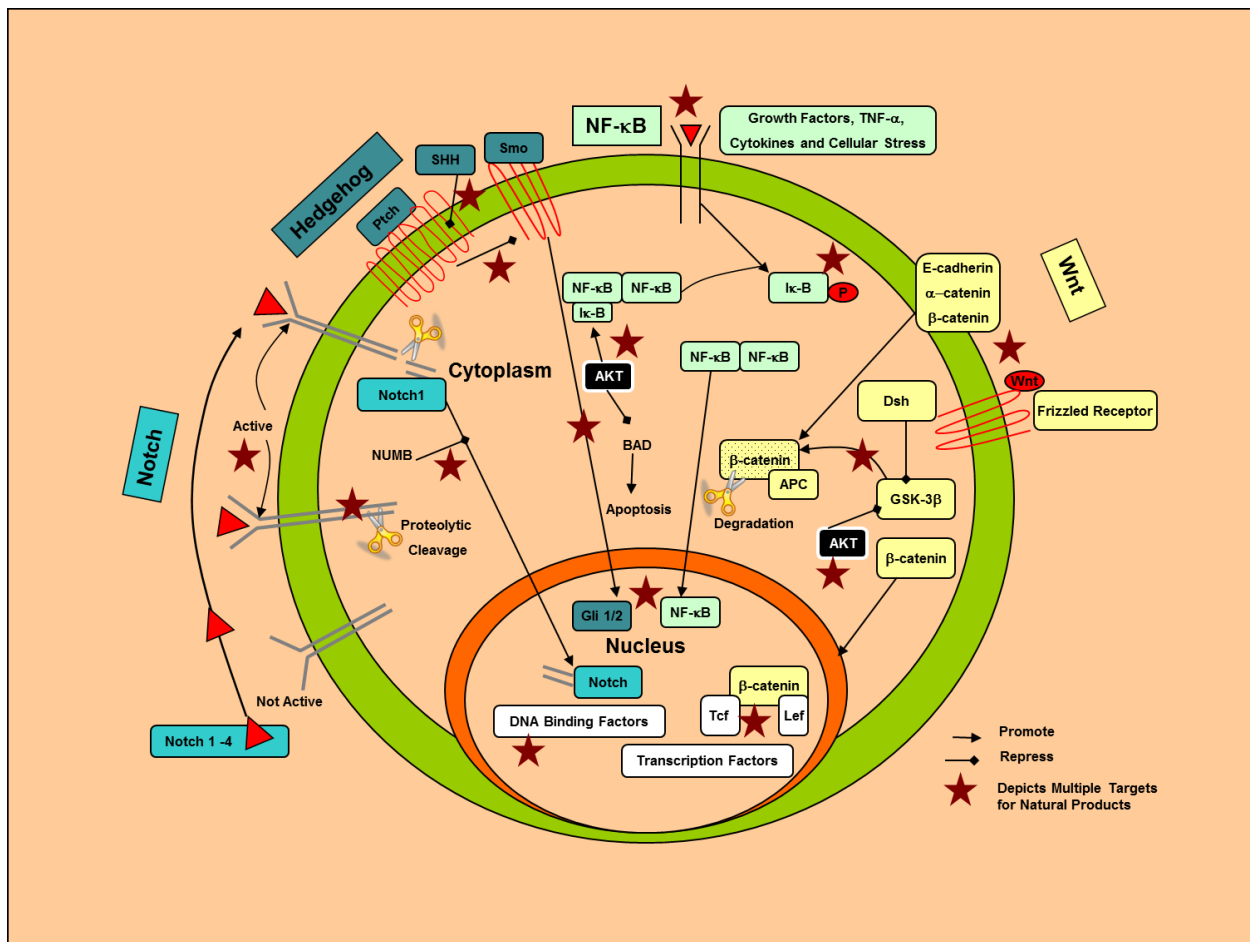
The lack of bioavailability of curcumin was known as a potential disadvantage for years and various strategies have been investigated to overcome the problem. One such strategy has been the use of piperine in combination of curcumin. Accordingly, concomitant administration of piperine and curcumin tends to increase the bioavailability (up to 2000%) compared to

administration of curcumin alone in an experimental group of people [421]. This finding could well be a possibility as piperine has been shown to inhibit P-glycoproteins and CYP3A4 expressed in enterocytes where the bioavailability of many orally ingested compounds are determined [422]. Alternatively, increasing the solubility of curcumin by heat as means of increasing the bio availability has been suggested [423]. This method is easily achievable and a well-cooked curry with tumeric and piperine could be a tasty way of obtaining the goodness of these natural compounds.

Based on the ability of curcumin and piperine to inhibit CSC's as described above, curcumin has great potential as a possible therapeutic agent against breast cancer. The majority of the breast cancer patients have tumors that respond to the naturally occurring hormone, estrogen. Therefore, most of the currently available drugs known to be effective against breast cancer can prevent the action of estrogen and are thus referred to as selective estrogen receptor modulators (SERM). Unfortunately, there is a cohort of patients whose tumours do not express estrogen receptor. SERMs are of no use for this group with ER -ve breast cancers. The potential of curcumin and piperine to suppress the self-renewal of stem cells could prove beneficial in ER +ve as well as ER -ve breast cancer patients.

## 10. Summary

Considering the existence of CSC's in breast cancer, and the inability of current therapeutic approaches to destroy such, we propose targeting CSC's as a tool to investigate the effect of natural dietary compounds. Specifically, we suggest isolates of soy and turmeric, and their effects on breast cancer tumors and metastasis reoccurrence in breast cancer. Similar to other developmental agents aimed at cancer signaling pathways, the optimal dosing, dosing regimens and adverse effects will have to be refined. However, these prospects are notably different than conventional therapeutics in several ways and such should be contemplated upon exploring such molecules. For example, consider the complex cross-talk of both inter- and intra-cell signaling pathways and how feedback mechanism effects may eliminate inhibitors via the actions of a single pathway. Figure 2 summarizes targeted areas of interruption via the major pathways involved in stem cell renewal as well as those pathways that may be responsible for downstream signaling to these major pathways. Additionally, if CSC's are primary targets, then metastasis incidence and/or cancer free survival may be a more appropriate efficacy endpoint in clinical trials than tumor volume. Perhaps then it would be more strategic in breast cancer to design preclinical trials based on incorporating combination regimens at the early stages of drug development while targeting multiple pathways and focusing on appropriate endpoints; all to avoid missing potentially important therapeutic benefits. While plant based derivatives, such as those considered in this chapter hold promise in breast cancer treatment and management, their development should be pursued systematically, guided by sound scientific principles.



**Figure 2.** (Tobin, GA, 2012) An Overview of the Major Signaling Networks Involved in Breast Cancer and CSC Self-renewal, including Notch, NFκB, Wnt and Hh. Symbols and acronyms have been discussed previously, with the exception of P = phosphorylation, BAD = Bcl-2-associated death promoter, and SHH = Sonic Hh. Also, NUMB references the Protein numb homolog that in humans is encoded by the NUMB gene.

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## References

- [1] Coughlin SS, Ekwueme DU. Breast cancer as a global health concern. *Cancer Epidemiol.* 2009 Nov;33(5):315-8. PubMed PMID: 19896917.
- [2] Canadian Cancer Statistics. 2012. Available at: <http://www.cancer.ca/en/cancer-information/cancer-101/cancer-statistics-at-a-glance/?region=on>
- [3] McClure C. Prince Edward Island Cancer Trends: 1980 - 2009. 2012 August. Document Publishing Center, Charlottetown, Prince Edward Island.
- [4] National Cancer Institute. SEER. Cancer Survival Statistics. 2009.
- [5] Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: markers and models. *Nat Rev Cancer.* 2005 Aug;5(8):591-602. PubMed PMID: 16056258.
- [6] Zhu S, Lee DA, Li S. IL-12 and IL-27 sequential gene therapy via intramuscular electroporation delivery for eliminating distal aggressive tumors. *J Immunol.* 2010 Mar 1;184(5):2348-54. PubMed PMID: 20139275.
- [7] Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* 2000 Jan 7;100(1):57-70. PubMed PMID: 10647931.
- [8] Pecorino L. *Molecular Biology of Cancer. Mechanisms, Targets and Therapeutics.* 2008 (Second Edition).
- [9] Merck Manual. Professional Edition. Ch. 253, Breast Cancer. Website: [http://www.merckmanuals.com/professional/gynecology\\_and\\_obstetrics/breast\\_disorders/breast\\_cancer.html](http://www.merckmanuals.com/professional/gynecology_and_obstetrics/breast_disorders/breast_cancer.html)
- [10] Porter RS, Justin L. Breast Cancer Merck Manual Professional Edition. 2010;Ch. 253.

- [11] Vernig B. Ductal carcinoma in situ of the breast: a systematic review of incidence, treatment and outcomes. *Journal of the National Cancer Institute*. 2010;102(3):170-8.
- [12] Sobin LH Wittekind GM. *TNM Classification of Malignant Tumors*. 2009;7th ed:310 pages.
- [13] Giuliano AE, Hunt KK, Ballman KV, Beitsch PD, Whitworth PW, Blumencranz PW, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA*. 2011 Feb 9;305(6):569-75. PubMed PMID: 21304082.
- [14] Marrazzo A, Taormina P, Gebbiab V, David M, Riili I, Lo Gerfo D, et al. Is sentinel lymph node biopsy more accurate than axillary dissection for staging nodal involvement in breast cancer patients? *Chir Ital*. 2007 Sep-Oct;59(5):693-9. PubMed PMID: 18019642.
- [15] Perou CM, Borresen-Dale AL. Systems biology and genomics of breast cancer. *Cold Spring Harbor perspectives in biology*. 2011 Feb;3(2). PubMed PMID: 21047916.
- [16] Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci U S A*. 1999 Aug 3;96(16):9212-7. PubMed PMID: 10430922.
- [17] Perou CM, Parker JS, Prat A, Ellis MJ, Bernard PS. Clinical implementation of the intrinsic subtypes of breast cancer. *Lancet Oncol*. 2010 Aug;11(8):718-9; 20-1. PubMed PMID: 20688274.
- [18] Perou CM. Molecular stratification of triple-negative breast cancers. *The oncologist*. 2011;16 Suppl 1:61-70. PubMed PMID: 21278442.
- [19] Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. *N Engl J Med*. 2009 Feb 19;360(8):790-800. PubMed PMID: 19228622.
- [20] Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol*. 2011 Feb;5(1):5-23. PubMed PMID: 21147047.
- [21] Ross JS. Multigene classifiers, prognostic factors, and predictors of breast cancer clinical outcome. *Adv Anat Pathol*. 2009 Jul;16(4):204-15. PubMed PMID: 19546609.
- [22] Herschkowitz JI, He X, Fan C, Perou CM. The functional loss of the retinoblastoma tumour suppressor is a common event in basal-like and luminal B breast carcinomas. *Breast Cancer Res*. 2008;10(5):R75. PubMed PMID: 18782450.
- [23] Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature*. 2000 Aug 17;406(6797):747-52. PubMed PMID: 10963602.
- [24] Perou CM. Molecular stratification of triple-negative breast cancers. *The Oncologist*. 2010;15 Suppl 5:39-48. PubMed PMID: 21138954.

- [25] Peppercorn J, Perou CM, Carey LA. Molecular subtypes in breast cancer evaluation and management: divide and conquer. *Cancer Invest.* 2008 Feb;26(1):1-10. PubMed PMID: 18181038.
- [26] Hu X, Stern HM, Ge L, O'Brien C, Haydu L, Honchell CD, et al. Genetic alterations and oncogenic pathways associated with breast cancer subtypes. *Mol Cancer Res.* 2009 Apr;7(4):511-22. PubMed PMID: 19372580.
- [27] Bild AH, Parker JS, Gustafson AM, Acharya CR, Hoadley KA, Anders C, et al. An integration of complementary strategies for gene-expression analysis to reveal novel therapeutic opportunities for breast cancer. *Breast Cancer Res.* 2009;11(4):R55. PubMed PMID: 19638211.
- [28] Hugh J, Hanson J, Cheang MC, Nielsen TO, Perou CM, Dumontet C, et al. Breast cancer subtypes and response to docetaxel in node-positive breast cancer: use of an immunohistochemical definition in the BCIRG 001 trial. *J Clin Oncol.* 2009 Mar 10;27(8):1168-76. PubMed PMID: 19204205.
- [29] Kwan ML, Kushi LH, Weltzien E, Maring B, Kutner SE, Fulton RS, et al. Epidemiology of breast cancer subtypes in two prospective cohort studies of breast cancer survivors. *Breast Cancer Res.* 2009;11(3):R31. PubMed PMID: 19463150.
- [30] Myal Y, Leygue E, Blanchard AA. Claudin 1 in breast tumorigenesis: revelation of a possible novel "claudin high" subset of breast cancers. *J Biomed Biotechnol.* 2010;2010:956897. PubMed PMID: 20490282.
- [31] Vargo-Gogola T, Rosen JM. Modelling breast cancer: one size does not fit all. *Nat Rev Cancer.* 2007 Sep;7(9):659-72. PubMed PMID: 17721431.
- [32] Nicholson RI, Johnston SR. Endocrine therapy--current benefits and limitations. *Breast Cancer Res Treat.* 2005;93 Suppl 1:S3-10. PubMed PMID: 16247594.
- [33] Rakha EA, Elsheikh SE, Aleskandarany MA, Habashi HO, Green AR, Powe DG, et al. Triple-negative breast cancer: distinguishing between basal and nonbasal subtypes. *Clin Cancer Res.* 2009 Apr 1;15(7):2302-10. PubMed PMID: 19318481.
- [34] Herschkowitz JI, Simin K, Weigman VJ, Mikaelian I, Usary J, Hu Z, et al. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome Biol.* 2007;8(5):R76. PubMed PMID: 17493263.
- [35] MacFarlane R SC, Masoudi H, Chia S. . Molecular changes in the primary breast cancer versus the relapsed/metastatic lesion from a large population based database and tissue microarray series. *J Clin Oncol.* 2008.;26(15S):1000.
- [36] Ravdin PM, Siminoff LA, Davis GJ, Mercer MB, Hewlett J, Gerson N, et al. Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer. *J Clin Oncol.* 2001 Feb 15;19(4):980-91. PubMed PMID: 11181660.

- [37] Olivotto IA, Bajdik CD, Ravdin PM, Speers CH, Coldman AJ, Norris BD, et al. Population-based validation of the prognostic model ADJUVANT! for early breast cancer. *J Clin Oncol*. 2005 Apr 20;23(12):2716-25. PubMed PMID: 15837986.
- [38] Silverstein MJ, Craig PH, Lagios MD, Waisman JK, Lewinsky BS, Colburn WJ, et al. Developing a prognostic index for ductal carcinoma in situ of the breast. Are we there yet? *Cancer*. 1996 Sep 1;78(5):1138-40. PubMed PMID: 8780555.
- [39] Silverstein MJ, Lagios MD, Craig PH, Waisman JR, Lewinsky BS, Colburn WJ, et al. A prognostic index for ductal carcinoma in situ of the breast. *Cancer*. 1996 Jun 1;77(11):2267-74. PubMed PMID: 8635094.
- [40] Dawson SJ, Makretsov N, Blows FM, Driver KE, Provenzano E, Le Quesne J, et al. BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. *Br J Cancer*. Aug 24;103(5):668-75. PubMed PMID: 20664598.
- [41] Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol*. 2005 Oct 1;23(28):7212-20. PubMed PMID: 16192605.
- [42] Hofseth LJ, Raafat AM, Osuch JR, Pathak DR, Slomski CA, Haslam SZ. Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast. *J Clin Endocrinol Metab*. 1999 Dec;84(12):4559-65. PubMed PMID: 10599719.
- [43] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol*. 2000 Mar;182(3):311-22. PubMed PMID: 10653597.
- [44] Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst*. 2007 Jan 17;99(2):167-70. PubMed PMID: 17228000.
- [45] Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and *in vitro* propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res*. 2005 Jul 1;65(13):5506-11. PubMed PMID: 15994920.
- [46] Polyak K. Breast cancer stem cells: a case of mistaken identity? *Stem Cell Rev*. 2007 Jun;3(2):107-9. PubMed PMID: 17873341.
- [47] Polyak K. Breast cancer: origins and evolution. *J Clin Invest*. 2007 Nov;117(11):3155-63. PubMed PMID: 17975657.
- [48] Simpson PT, Reis-Filho JS, Gale T, Lakhani SR. Molecular evolution of breast cancer. *J Pathol*. 2005 Jan;205(2):248-54. PubMed PMID: 15641021.
- [49] Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer*. 2003 Dec;3(12):895-902. PubMed PMID: 14737120.

- [50] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A*. 2003 Apr 1;100(7):3983-8. PubMed PMID: 12629218.
- [51] Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res*. 2006 Jun 15;66(12):6063-71. PubMed PMID: 16778178.
- [52] Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell stem cell*. 2007 Nov;1(5):555-67. PubMed PMID: 18371393.
- [53] Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, et al. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. *Cancer Res*. 2009 Feb 15;69(4):1302-13. PubMed PMID: 19190339. Pubmed Central PMCID: 2819227.
- [54] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008 May 16;133(4):704-15. PubMed PMID: 18485877. Pubmed Central PMCID: 2728032.
- [55] Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997 Jul;3(7):730-7. PubMed PMID: 9212098.
- [56] Singh SM, Murphy B, O'Reilly RL. Involvement of gene-diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia. *Clin Genet*. 2003 Dec;64(6):451-60. PubMed PMID: 14986824.
- [57] Yang DG, Liu L, Zheng XY. Cyclin-dependent kinase inhibitor p16(INK4a) and telomerase may co-modulate endothelial progenitor cells senescence. *Ageing Res Rev*. 2008 Apr;7(2):137-46. PubMed PMID: 18343732.
- [58] Yang W, Lam P, Kitching R, Kahn HJ, Yee A, Aubin JE, et al. Breast cancer metastasis in a human bone NOD/SCID mouse model. *Cancer Biol Ther*. 2007 Aug;6(8):1289-94. PubMed PMID: 17704641.
- [59] Hambardzumyan D, Squatrito M, Holland EC. Radiation resistance and stem-like cells in brain tumors. *Cancer Cell*. 2006 Dec;10(6):454-6. PubMed PMID: 17157785.
- [60] Korkaya H, Paulson A, Charafe-Jauffret E, Ginestier C, Brown M, Dutcher J, et al. Regulation of mammary stem/progenitor cells by PTEN/Akt/beta-catenin signaling. *PLoS Biol*. 2009 Jun 2;7(6):e1000121. PubMed PMID: 19492080.
- [61] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001 Nov 1;414(6859):105-11. PubMed PMID: 11689955.



- [62] Shafee N, Smith CR, Wei S, Kim Y, Mills GB, Hortobagyi GN, et al. Cancer stem cells contribute to cisplatin resistance in Brca1/p53-mediated mouse mammary tumors. *Cancer Res.* 2008 May 1;68(9):3243-50. PubMed PMID: 18451150.
- [63] Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. *Nat Rev Drug Discov.* 2009 Oct;8(10):806-23. PubMed PMID: 19794444.
- [64] Bapat SA. Evolution of cancer stem cells. *Semin Cancer Biol.* 2007 Jun;17(3):204-13. PubMed PMID: 16787749.
- [65] Han JS, Crowe DL. Tumor initiating cancer stem cells from human breast cancer cell lines. *Int J Oncol.* 2009 May;34(5):1449-53. PubMed PMID: 19360358.
- [66] Miller SJ, Lavker RM, Sun TT. Interpreting epithelial cancer biology in the context of stem cells: tumor properties and therapeutic implications. *Biochim Biophys Acta.* 2005 Sep 25;1756(1):25-52. PubMed PMID: 16139432.
- [67] Allred DC, Wu Y, Mao S, Nagtegaal ID, Lee S, Perou CM, et al. Ductal carcinoma in situ and the emergence of diversity during breast cancer evolution. *Clin Cancer Res.* 2008 Jan 15;14(2):370-8. PubMed PMID: 18223211.
- [68] Hu M, Yao J, Carroll DK, Weremowicz S, Chen H, Carrasco D, et al. Regulation of in situ to invasive breast carcinoma transition. *Cancer Cell.* 2008 May;13(5):394-406. PubMed PMID: 18455123.
- [69] Klein CA. Cancer. The metastasis cascade. *Science.* 2008 Sep 26;321(5897):1785-7. PubMed PMID: 18818347.
- [70] Husemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, et al. Systemic spread is an early step in breast cancer. *Cancer Cell.* 2008 Jan;13(1):58-68. PubMed PMID: 18167340.
- [71] Ansieau S, Hinkal G, Thomas C, Bastid J, Puisieux A. Early origin of cancer metastases: dissemination and evolution of premalignant cells. *Cell cycle.* 2008 Dec;7(23):3659-63. PubMed PMID: 19029812.
- [72] Podsypanina K, Du YC, Jechlinger M, Beverly LJ, Hambardzumyan D, Varmus H. Seeding and propagation of untransformed mouse mammary cells in the lung. *Science.* 2008 Sep 26;321(5897):1841-4. PubMed PMID: 18755941.
- [73] Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, Norton L, et al. Tumor self-seeding by circulating cancer cells. *Cell.* 2009 Dec 24;139(7):1315-26. PubMed PMID: 20064377.
- [74] Carroll J. Breast cancer breakthrough spotlights sequencing impact. *Fierce Biotech Research.* 2009.
- [75] Ahmed M, Lalloo F, Evans DG. Update on genetic predisposition to breast cancer. *Expert Rev Anticancer Ther.* 2009 Aug;9(8):1103-13. PubMed PMID: 19671030.

- [76] Lynch HT, Marcus JN, Rubinstein WS. Stemming the tide of cancer for BRCA1/2 mutation carriers. *J Clin Oncol*. 2008 Sep 10;26(26):4239-43. PubMed PMID: 18779610.
- [77] Thompson ME. BRCA1 16 years later: an overview. 2010 Aug;277(15):3071. PubMed PMID: 20608973.
- [78] Walsh T, Casadei S, Coats KH, Swisher E, Stray SM, Higgins J, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. *Jama*. 2006 Mar 22;295(12):1379-88. PubMed PMID: 16551709.
- [79] Campeau PM, Foulkes WD, Tischkowitz MD. Hereditary breast cancer: new genetic developments, new therapeutic avenues. *Hum Genet*. 2008 Aug;124(1):31-42. PubMed PMID: 18575892.
- [80] Hashizume T, Fukuda T, Nagaoka T, Tada H, Yamada H, Watanabe K, et al. Cell type dependent endocytic internalization of ErbB2 with an artificial peptide ligand that binds to ErbB2. *Cell Biol Int*. 2008 Jul;32(7):814-26. PubMed PMID: 18442934.
- [81] Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature*. 1997 Apr 24;386(6627):761, 3. PubMed PMID: 9126728.
- [82] Hall J, Montesano R. DNA alkylation damage: consequences and relevance to tumour production. *Mutat Res*. 1990 Nov-Dec;233(1-2):247-52. PubMed PMID: 2233806.
- [83] Hall JG. Genomic imprinting: review and relevance to human diseases. *Am J Hum Genet*. 1990 May;46(5):857-73. PubMed PMID: 2187341.
- [84] Rosnoble C, Vischer UM, Gerard RD, Irminger JC, Halban PA, Kruithof EK. Storage of tissue-type plasminogen activator in Weibel-Palade bodies of human endothelial cells. *Arterioscler Thromb Vasc Biol*. 1999 Jul;19(7):1796-803. PubMed PMID: 10397700.
- [85] Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*. 1994 Oct 7;266(5182):66-71. PubMed PMID: 7545954.
- [86] Starita LM, Parvin JD. The multiple nuclear functions of BRCA1: transcription, ubiquitination and DNA repair. *Curr Opin Cell Biol*. 2003 Jun;15(3):345-50. PubMed PMID: 12787778.
- [87] Duncan JA, Reeves JR, Cooke TG. BRCA1 and BRCA2 proteins: roles in health and disease. *Mol Pathol*. 1998 Oct;51(5):237-47. PubMed PMID: 10193517.
- [88] Yoshida K, Toge T. [Telomerase activity in gastrointestinal, bladder and breast carcinomas and their clinical applications]. *Nihon Rinsho*. 2004 Jul;62(7):1368-76. PubMed PMID: 15283158.
- [89] Rotman G, Shiloh Y. ATM: from gene to function. *Hum Mol Genet*. 1998;7(10):1555-63. PubMed PMID: 9735376.

- [90] Thompson D, Antoniou AC, Jenkins M, Marsh A, Chen X, Wayne T, et al. Two ATM variants and breast cancer risk. *Hum Mutat.* 2005 Jun;25(6):594-5. PubMed PMID: 15880680.
- [91] Antoniou AC, Easton DF. Models of genetic susceptibility to breast cancer. *Oncogene.* 2006 Sep 25;25(43):5898-905. PubMed PMID: 16998504.
- [92] Hoekstra MF. Responses to DNA damage and regulation of cell cycle checkpoints by the ATM protein kinase family. *Curr Opin Genet Dev.* 1997 Apr;7(2):170-5. PubMed PMID: 9115420.
- [93] Xu Y, Baltimore D. Dual roles of ATM in the cellular response to radiation and in cell growth control. *Genes Dev.* 1996 Oct 1;10(19):2401-10. PubMed PMID: 8843193.
- [94] Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. *Cancer Res.* 1994 Mar 1;54(5):1298-304. PubMed PMID: 8118819.
- [95] Li FP, Correa P, Fraumeni JF, Jr. Testing for germ line p53 mutations in cancer families. *Cancer Epidemiol Biomarkers Prev.* 1991 Nov-Dec;1(1):91-4. PubMed PMID: 1845175.
- [96] Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science.* 1990 Nov 30;250(4985):1233-8. PubMed PMID: 1978757.
- [97] Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science.* 2000 Mar 10;287(5459):1824-7. PubMed PMID: 10710310.
- [98] Lee JM, Lee YC, Yang SY, Shi WL, Lee CJ, Luh SP, et al. Genetic polymorphisms of p53 and GSTP1, but not NAT2, are associated with susceptibility to squamous-cell carcinoma of the esophagus. *Int J Cancer.* 2000 Sep 20;89(5):458-64. PubMed PMID: 11008209.
- [99] Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science.* 1998 Dec 4;282(5395):1893-7. PubMed PMID: 9836640.
- [100] Chu EC, Tarnawski AS. PTEN regulatory functions in tumor suppression and cell biology. *Med Sci Monit.* 2004 Oct;10(10):RA235-41. PubMed PMID: 15448614.
- [101] Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet.* 1997 Apr;15(4):356-62. PubMed PMID: 9090379.
- [102] Huntsman DG, Chin SF, Muleris M, Batley SJ, Collins VP, Wiedemann LM, et al. MLL2, the second human homolog of the *Drosophila trithorax* gene, maps to 19q13.1

and is amplified in solid tumor cell lines. *Oncogene*. 1999 Dec 23;18(56):7975-84. PubMed PMID: 10637508.

- [103] Semb H, Christofori G. The tumor-suppressor function of E-cadherin. *Am J Hum Genet*. 1998 Dec;63(6):1588-93. PubMed PMID: 9837810.
- [104] Wong AS, Gumbiner BM. Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. *J Cell Biol*. 2003 Jun 23;161(6):1191-203. PubMed PMID: 12810698.
- [105] Desmedt C, Haibe-Kains B, Wirapati P, Buyse M, Larsimont D, Bontempi G, et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin Cancer Res*. 2008 Aug 15;14(16):5158-65. PubMed PMID: 18698033.
- [106] Lange CA. Challenges to defining a role for progesterone in breast cancer. *Steroids*. 2008 Oct;73(9-10):914-21. PubMed PMID: 18243264.
- [107] Lange CA, Richer JK, Horwitz KB. Hypothesis: Progesterone primes breast cancer cells for cross-talk with proliferative or antiproliferative signals. *Mol Endocrinol*. 1999 Jun;13(6):829-36. PubMed PMID: 10379882.
- [108] Fang H, Tong W, Shi LM, Blair R, Perkins R, Branham W, et al. Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. *Chem Res Toxicol*. 2001 Mar;14(3):280-94. PubMed PMID: 11258977.
- [109] Lin C-Y, SO. Discovery of estrogen receptor alpha target genes and response elements in breast tumor cells. *Genome Biol*. 2004 5(9):R66(PMID- 15345050).
- [110] Dowsett M, Houghton J, Iden C, Salter J, Farndon J, A'Hern R, et al. Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. *Ann Oncol*. 2006 May; 17(5):818-26. PubMed PMID: 16497822.
- [111] Miller K. *Choices in Breast Cancer Treatment*. 2008. ISBN-10: 0801886848 ISBN-13: 978-0801886843.
- [112] Lupulescu A. Estrogen use and cancer incidence: a review. *Cancer Invest*. 1995;13(3): 287-95. PubMed PMID: 7743382.
- [113] Cummings SR, Duong T, Kenyon E, Cauley JA, Whitehead M, Krueger KA. Serum estradiol level and risk of breast cancer during treatment with raloxifene. *Jama*. 2002 Jan 9;287(2):216-20. PubMed PMID: 11779264.
- [114] Panico S, Pisani P, Muti P, Recchione C, Cavalleri A, Totis A, et al. Diurnal variation of testosterone and estradiol: a source of bias in comparative studies on breast cancer. *J Endocrinol Invest*. 1990 May;13(5):423-6. PubMed PMID: 2380505.

- [115] Tesarik J, Garrigosa L, Mendoza C. Estradiol modulates breast cancer cell apoptosis: a novel nongenomic steroid action relevant to carcinogenesis. *Steroids*. 1999 Jan-Feb; 64(1-2):22-7. PubMed PMID: 10323669.
- [116] Ahmed MI, Lennard TW. Breast cancer: role of neoadjuvant therapy. *Int J Surg*. 2009 Oct;7(5):416-20. PubMed PMID: 19524705.
- [117] Weber BL, Nathanson KL. Low penetrance genes associated with increased risk for breast cancer. *Eur J Cancer*. 2000 Jun;36(10):1193-9. PubMed PMID: 10882856.
- [118] Kurzer MS. Hormonal effects of soy in premenopausal women and men. *J Nutr*. 2002 Mar;132(3):570S-3S. PubMed PMID: 11880595.
- [119] Lasfargues EY OL. Cultivation of human breast carcinomas. *J Natl Cancer Inst*. 1958; 21:1131-47.
- [120] Lacroix M, Leclercq G. Relevance of breast cancer cell lines as models for breast tumours: an update. *Breast Cancer Res Treat*. 2004 Feb;83(3):249-89. PubMed PMID: 14758095.
- [121] Fillmore C, Kuperwasser C. Human breast cancer stem cell markers CD44 and CD24: enriching for cells with functional properties in mice or in man? *Breast Cancer Res*. 2007;9(3):303. PubMed PMID: 17540049.
- [122] Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L, et al. Reconstruction of functionally normal and malignant human breast tissues in mice. *Proc Natl Acad Sci U S A*. 2004 Apr 6;101(14):4966-71. PubMed PMID: 15051869.
- [123] Radisky DC, Bissell MJ. Cancer. Respect thy neighbor! *Science*. 2004 Feb 6;303(5659): 775-7. PubMed PMID: 14764858.
- [124] Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS. Stem cells in normal breast development and breast cancer. *Cell Prolif*. 2003 Oct;36 Suppl 1:59-72. PubMed PMID: 14521516.
- [125] Rae JM, Ramus SJ, Waltham M, Armes JE, Campbell IG, Clarke R, et al. Common origins of MDA-MB-435 cells from various sources with those shown to have melanoma properties. *Clinical & experimental metastasis*. 2004;21(6):543-52. PubMed PMID: 15679052.
- [126] Rae JM, Creighton CJ, Meck JM, Haddad BR, Johnson MD. MDA-MB-435 cells are derived from M14 melanoma cells--a loss for breast cancer, but a boon for melanoma research. *Breast Cancer Res Treat*. 2007 Jul;104(1):13-9. PubMed PMID: 17004106.
- [127] Ellison G, Klinowska T, Westwood RF, Docter E, French T, Fox JC. Further evidence to support the melanocytic origin of MDA-MB-435. *Mol Pathol*. 2002 Oct;55(5):294-9. PubMed PMID: 12354931. Pubmed Central PMCID: 1187258.

- [128] Christgen M, Lehmann U. MDA-MB-435: the questionable use of a melanoma cell line as a model for human breast cancer is ongoing. *Cancer Biol Ther.* 2007 Sep;6(9):1355-7. PubMed PMID: 17786032.
- [129] Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell.* 2006 Dec;10(6):515-27. PubMed PMID: 17157791.
- [130] Hollestelle A NJ, Smid M, Lam S, Elstrodt F, Wasielewski M, Ng SS, French PJ PJ, Rozendaal MJ, Riaz M, Koopman DG, Ten Hagen TL, de Leeuw BH ZE, Teunisse A, van der Spek PJ, Klijn JG, Dinjens WN, Ethier SP CH, Jochemsen AG, den Bakker MA, Foekens JA, Martens JW, M S. : Distinct gene mutation profiles among luminal-type and basaltype breast cancer cell lines. *Breast Cancer Res Treat.* 2010; 121:53-64.
- [131] Koch M, Hussein F, Woeste A, Grundker C, Frontzek K, Emons G, et al. CD36-mediated activation of endothelial cell apoptosis by an N-terminal recombinant fragment of thrombospondin-2 inhibits breast cancer growth and metastasis *in vivo*. *Breast Cancer Res Treat.* 2011 Jul;128(2):337-46. PubMed PMID: 20714802. Pubmed Central PMCID: 3291836.
- [132] Bachmeier B, Fichtner I, Killian PH, Kronski E, Pfeffer U, Efferth T. Development of resistance towards artesunate in MDA-MB-231 human breast cancer cells. *PLoS One.* 2011;6(5):e20550. PubMed PMID: 21637790. Pubmed Central PMCID: 3102747.
- [133] Holliday DL, Speirs V. Choosing the right cell line for breast cancer research. *Breast Cancer Res.* 2011;13(4):215. PubMed PMID: 21884641. Pubmed Central PMCID: 3236329.
- [134] Eccles SA, Welch DR. Metastasis: recent discoveries and novel treatment strategies. *Lancet.* 2007 May 19;369(9574):1742-57. PubMed PMID: 17512859.
- [135] Vernon AE, Bakewell SJ, Chodosh LA. Deciphering the molecular basis of breast cancer metastasis with mouse models. *Rev Endocr Metab Disord.* 2007 Sep;8(3):199-213. PubMed PMID: 17657606.
- [136] Kim IS, Baek SH. Mouse models for breast cancer metastasis. *Biochem Biophys Res Commun.* 2010 Apr 9;394(3):443-7. PubMed PMID: 20230796.
- [137] Jonkers J, Derksen PW. Modeling metastatic breast cancer in mice. *J Mammary Gland Biol Neoplasia.* 2007 Sep;12(2-3):191-203. PubMed PMID: 17587153.
- [138] Maglione JE, Moghanaki D, Young LJ, Manner CK, Ellies LG, Joseph SO, et al. Transgenic Polyoma middle-T mice model premalignant mammary disease. *Cancer Res.* 2001 Nov 15;61(22):8298-305. PubMed PMID: 11719463.
- [139] Blackburn AC, Jerry DJ. Knockout and transgenic mice of Trp53: what have we learned about p53 in breast cancer? *Breast Cancer Res.* 2002;4(3):101-11. PubMed PMID: 12052252.

- [140] Ye Y, Qiu TH, Kavanaugh C, Green JE. Molecular mechanisms of breast cancer progression: lessons from mouse mammary cancer models and gene expression profiling. *Breast Dis.* 2004;19:69-82. PubMed PMID: 15687699.
- [141] Julien SG, Dube N, Read M, Penney J, Paquet M, Han Y, et al. Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and protects from lung metastasis. *Nat Genet.* 2007 Mar;39(3):338-46. PubMed PMID: 17259984.
- [142] Maroulakou IG, Oemler W, Naber SP, Tschlis PN. Akt1 ablation inhibits, whereas Akt2 ablation accelerates, the development of mammary adenocarcinomas in mouse mammary tumor virus (MMTV)-ErbB2/neu and MMTV-polyoma middle T transgenic mice. *Cancer Res.* 2007 Jan 1;67(1):167-77. PubMed PMID: 17210696.
- [143] DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell.* 2009 Aug 4;16(2):91-102. PubMed PMID: 19647220.
- [144] Sharpless NE, Depinho RA. The mighty mouse: genetically engineered mouse models in cancer drug development. *Nat Rev Drug Discov.* 2006 Sep;5(9):741-54. PubMed PMID: 16915232.
- [145] Lin SC, Lee KF, Nikitin AY, Hilsenbeck SG, Cardiff RD, Li A, et al. Somatic mutation of p53 leads to estrogen receptor alpha-positive and -negative mouse mammary tumors with high frequency of metastasis. *Cancer Res.* 2004 May 15;64(10):3525-32. PubMed PMID: 15150107.
- [146] Fantozzi A, Christofori G. Mouse models of breast cancer metastasis. *Breast Cancer Res.* 2006;8(4):212. PubMed PMID: 16887003.
- [147] Aslakson CJ, Miller FR. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Res.* 1992 Mar 15;52(6):1399-405. PubMed PMID: 1540948.
- [148] Pulaski BA, Terman DS, Khan S, Muller E, Ostrand-Rosenberg S. Cooperativity of Staphylococcal aureus enterotoxin B superantigen, major histocompatibility complex class II, and CD80 for immunotherapy of advanced spontaneous metastases in a clinically relevant postoperative mouse breast cancer model. *Cancer Res.* 2000 May 15;60(10):2710-5. PubMed PMID: 10825145.
- [149] Pulaski BA, Ostrand-Rosenberg S. Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. *Cancer Res.* 1998 Apr 1;58(7):1486-93. PubMed PMID: 9537252.

- [150] Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*. 2004 Jun 25;117(7):927-39. PubMed PMID: 15210113.
- [151] Hurst J, Maniar N, Tombarkiewicz J, Lucas F, Roberson C, Steplewski Z, et al. A novel model of a metastatic human breast tumour xenograft line. *Br J Cancer*. 1993 Aug; 68(2):274-6. PubMed PMID: 8394103.
- [152] Fogh J, Giovanella BC. *The Nude Mouse in Experimental and Clinical Research*. Academic Press; 1982.
- [153] Schuler W, Bosma MJ. Nature of the scid defect: a defective VDJ recombinase system. *Current topics in microbiology and immunology*. 1989;152:55-62. PubMed PMID: 2805798.
- [154] Dewan MZ, Terunuma H, Ahmed S, Ohba K, Takada M, Tanaka Y, et al. Natural killer cells in breast cancer cell growth and metastasis in SCID mice. *Biomed Pharmacother*. 2005 Oct;59 Suppl 2:S375-9. PubMed PMID: 16507413.
- [155] Ottewell PD, Coleman RE, Holen I. From genetic abnormality to metastases: murine models of breast cancer and their use in the development of anticancer therapies. *Breast Cancer Res Treat*. 2006 Mar;96(2):101-13. PubMed PMID: 16319986.
- [156] Lu X, Kang Y. Metalloproteinases and osteoblast EGFR signaling in osteolytic bone metastasis of breast cancer. *Cell Cycle*. 2009 Dec;8(23):3804-5. PubMed PMID: 19934661.
- [157] Alonso DF, Farias EF, Urtreger A, Ladedá V, Vidal MC, Bal De Kier Joffe E. Characterization of F3II, a sarcomatoid mammary carcinoma cell line originated from a clonal subpopulation of a mouse adenocarcinoma. *Journal of Surgical Oncology*. 1996 Aug;62(4):288-97. PubMed PMID: 8691844.
- [158] Alonso DF, Farina HG, Skilton G, Gabri MR, De Lorenzo MS, Gomez DE. Reduction of mouse mammary tumor formation and metastasis by lovastatin, an inhibitor of the mevalonate pathway of cholesterol synthesis. *Breast Cancer Res Treat*. 1998 Jul; 50(1):83-93. PubMed PMID: 9802623.
- [159] Simstein R, Burow M, Parker A, Weldon C, Beckman B. Apoptosis, chemoresistance, and breast cancer: insights from the MCF-7 cell model system. *Exp Biol Med (Maywood)*. 2003 Oct;228(9):995-1003. PubMed PMID: 14530507.
- [160] Levenson AS, Jordan VC. MCF-7: the first hormone-responsive breast cancer cell line. *Cancer Res*. 1997 Aug 1;57(15):3071-8. PubMed PMID: 9242427.
- [161] Soule HD, Vazquez J, Long A, Albert S, Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. *J Natl Cancer Inst*. 1973 Nov;51(5):1409-16. PubMed PMID: 4357757.



- [162] Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. *Nature*. 2009 Jun 18;459(7249):1005-9. PubMed PMID: 19421193.
- [163] Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, et al. Genes that mediate breast cancer metastasis to lung. *Nature*. 2005 Jul 28;436(7050):518-24. PubMed PMID: 16049480.
- [164] Qi Q, Gu H, Yang Y, Lu N, Zhao J, Liu W, et al. Involvement of matrix metalloproteinase 2 and 9 in gambogic acid induced suppression of MDA-MB-435 human breast carcinoma cell lung metastasis. *J Mol Med*. 2008 Dec;86(12):1367-77. PubMed PMID: 18777017.
- [165] Charafe-Jauffret E, Ginestier C, Birnbaum D. Breast cancer stem cells: tools and models to rely on. *BMC Cancer*. 2009;9:202. PubMed PMID: 19555472.
- [166] Elstrodt F, Hollestelle A, Nagel JH, Gorin M, Wasielewski M, van den Ouweland A, et al. BRCA1 mutation analysis of 41 human breast cancer cell lines reveals three new deleterious mutants. *Cancer Res*. 2006 Jan 1;66(1):41-5. PubMed PMID: 16397213.
- [167] Kuperwasser C, Dessain S, Bierbaum BE, Garnet D, Sperandio K, Gauvin GP, et al. A mouse model of human breast cancer metastasis to human bone. *Cancer Res*. 2005 Jul 15;65(14):6130-8. PubMed PMID: 16024614.
- [168] Sotoca AM, Gelpke MD, Boeren S, Strom A, Gustafsson JA, Murk AJ, et al. Quantitative proteomics and transcriptomics addressing the estrogen receptor subtype-mediated effects in T47D breast cancer cells exposed to the phytoestrogen genistein. *Mol Cell Proteomics*. 2010 Jan;10(1):M110 002170. PubMed PMID: 20884965.
- [169] Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res*. 1998 Jul 1;58(13):2825-31. PubMed PMID: 9661897.
- [170] Sporn JR, Bilgrami SA. Weekly paclitaxel plus Herceptin in metastatic breast cancer patients who relapse after stem-cell transplant. *Ann Oncol*. 1999 Oct;10(10):1259-60. PubMed PMID: 10586349.
- [171] Davis AL, Klitus M, Mintzer DM. Chemotherapy-induced amenorrhea from adjuvant breast cancer treatment: the effect of the addition of taxanes. *Clin Breast Cancer*. 2005 Dec;6(5):421-4. PubMed PMID: 16381625.
- [172] Semenza GL. A new weapon for attacking tumor blood vessels. *N Engl J Med*. 2008 May 8;358(19):2066-7. PubMed PMID: 18463385.
- [173] Pollack A. FDA Rejects Use of Drugs in Breast Cancer. 2010; December 16. Available at: [http://www.nytimes.com/2010/12/17/health/policy/17drug.html?\\_r=0](http://www.nytimes.com/2010/12/17/health/policy/17drug.html?_r=0)

- [174] Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004 Jun 3;350(23):2335-42. PubMed PMID: 15175435.
- [175] Mateos MV, Hernandez JM, Hernandez MT, Gutierrez NC, Palomera L, Fuertes M, et al. Bortezomib plus melphalan and prednisone in elderly untreated patients with multiple myeloma: results of a multicenter phase 1/2 study. *Blood*. 2006 Oct 1;108(7):2165-72. PubMed PMID: 16772605.
- [176] Mitsiades N, Mitsiades CS, Richardson PG, Poulaki V, Tai YT, Chauhan D, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. *Blood*. 2003 Mar 15;101(6):2377-80. PubMed PMID: 12424198.
- [177] Moreau P, Hulin C, Facon T. Frontline treatment of multiple myeloma in elderly patients. *Blood Rev*. 2008 Nov;22(6):303-9. PubMed PMID: 18550234.
- [178] Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med*. 2003 Jul 31;349(5):427-34. PubMed PMID: 12890841.
- [179] Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature*. 2005 Dec 8;438(7069):820-7. PubMed PMID: 16341007.
- [180] Radloff DR, Rinella ES, Threadgill DW. Modeling cancer patient populations in mice: complex genetic and environmental factors. *Drug Discov Today Dis Models*. 2008;4(2):83-8. PubMed PMID: 19122874.
- [181] Prendergast GC, Jaffee EM. Cancer immunologists and cancer biologists: why we didn't talk then but need to now. *Cancer Res*. 2007 Apr 15;67(8):3500-4. PubMed PMID: 17413003.
- [182] Burstein HJ. The distinctive nature of HER2-positive breast cancers. *N Engl J Med*. 2005 Oct 20;353(16):1652-4. PubMed PMID: 16236735.
- [183] Hansen RK, Bissell MJ. Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones. *Endocr Relat Cancer*. 2000 Jun;7(2):95-113. PubMed PMID: 10903527.
- [184] Hiscox SG, Julia: Nicholson, I. . Therapeutic Resistance to Anti-Hormonal Drugs in Breast Cancer. *New Molecular Aspects and their Potential as Targets*. 2009:87.
- [185] Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. *Breast Cancer Res*. 2004;6(6):R605-15. PubMed PMID: 15535842.

- [186] Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. *Breast Cancer Res.* 2005;7(3):86-95. PubMed PMID: 15987436.
- [187] Shostak K, Chariot A. NF-kappaB, stem cells and breast cancer: the links get stronger. *Breast Cancer Res.* 2011;13(4):214. PubMed PMID: 21867572. Pubmed Central PMCID: 3236328.
- [188] Mumm JS, Kopan R. Notch signaling: from the outside in. *Dev Biol.* 2000 Dec 15;228(2):151-65. PubMed PMID: 11112321.
- [189] Li Y, Wicha MS, Schwartz SJ, Sun D. Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds. *The Journal of nutritional biochemistry.* 2011 Sep;22(9):799-806. PubMed PMID: 21295962. Pubmed Central PMCID: 3248810.
- [190] Borggreffe T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. *Cell Mol Life Sci.* 2009 May;66(10):1631-46. PubMed PMID: 19165418.
- [191] Wu JY, Rao Y. Fringe: defining borders by regulating the notch pathway. *Curr Opin Neurobiol.* 1999 Oct;9(5):537-43. PubMed PMID: 10508746.
- [192] Oswald F, Liptay S, Adler G, Schmid RM. NF-kappaB2 is a putative target gene of activated Notch-1 via RBP-Jkappa. *Mol Cell Biol.* 1998 Apr;18(4):2077-88. PubMed PMID: 9528780.
- [193] Palomero T, Lim WK, Odom DT, Sulis ML, Real PJ, Margolin A, et al. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. *Proc Natl Acad Sci U S A.* 2006 Nov 28;103(48):18261-6. PubMed PMID: 17114293.
- [194] Rangarajan A, Talora C, Okuyama R, Nicolas M, Mammucari C, Oh H, et al. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO J.* 2001 Jul 2;20(13):3427-36. PubMed PMID: 11432830.
- [195] Ronchini C, Capobianco AJ. Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): implication for cell cycle disruption in transformation by Notch(ic). *Mol Cell Biol.* 2001 Sep;21(17):5925-34. PubMed PMID: 11486031.
- [196] Satoh Y, Matsumura I, Tanaka H, Ezoe S, Sugahara H, Mizuki M, et al. Roles for c-Myc in self-renewal of hematopoietic stem cells. *J Biol Chem.* 2004 Jun 11;279(24):24986-93. PubMed PMID: 15067010.
- [197] Weng AP, Millholland JM, Yashiro-Ohtani Y, Arcangeli ML, Lau A, Wai C, et al. c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. *Genes Dev.* 2006 Aug 1;20(15):2096-109. PubMed PMID: 16847353.
- [198] Wang Z, Li Y, Banerjee S, Sarkar FH. Emerging role of Notch in stem cells and cancer. *Cancer Lett.* 2009 Jun 28;279(1):8-12. PubMed PMID: 19022563.

- [199] Kakarala M, Wicha MS. Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy. *J Clin Oncol.* 2008 Jun 10;26(17):2813-20. PubMed PMID: 18539959.
- [200] Peacock CD, Watkins DN. Cancer stem cells and the ontogeny of lung cancer. *J Clin Oncol.* 2008 Jun 10;26(17):2883-9. PubMed PMID: 18539968.
- [201] Scoville DH, Sato T, He XC, Li L. Current view: intestinal stem cells and signaling. *Gastroenterology.* 2008 Mar;134(3):849-64. PubMed PMID: 18325394.
- [202] Wilson A, Radtke F. Multiple functions of Notch signaling in self-renewing organs and cancer. *FEBS Lett.* 2006 May 22;580(12):2860-8. PubMed PMID: 16574107.
- [203] Dickson BC, Mulligan AM, Zhang H, Lockwood G, O'Malley FP, Egan SE, et al. High-level JAG1 mRNA and protein predict poor outcome in breast cancer. *Mod Pathol.* 2007 Jun;20(6):685-93. PubMed PMID: 17507991.
- [204] Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR, et al. High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. *Cancer Res.* 2005 Sep 15;65(18):8530-7. PubMed PMID: 16166334.
- [205] Rizzo P, Miao H, D'Souza G, Osipo C, Song LL, Yun J, et al. Cross-talk between notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches. *Cancer Res.* 2008 Jul 1;68(13):5226-35. PubMed PMID: 18593923.
- [206] Farnie G, Clarke RB. Mammary stem cells and breast cancer-role of Notch signalling. *Stem Cell Rev.* 2007 Jun;3(2):169-75. PubMed PMID: 17873349.
- [207] Farnie G, Clarke RB, Spence K, Pinnock N, Brennan K, Anderson NG, et al. Novel cell culture technique for primary ductal carcinoma in situ: role of Notch and epidermal growth factor receptor signaling pathways. *J Natl Cancer Inst.* 2007 Apr 18;99(8):616-27. PubMed PMID: 17440163.
- [208] Sansone P, Storci G, Giovannini C, Pandolfi S, Pianetti S, Taffurelli M, et al. p66Shc/Notch-3 interplay controls self-renewal and hypoxia survival in human stem/progenitor cells of the mammary gland expanded *in vitro* as mammospheres. *Stem Cells.* 2007 Mar;25(3):807-15. PubMed PMID: 17158237.
- [209] Sansone P, Storci G, Tavolari S, Guarnieri T, Giovannini C, Taffurelli M, et al. IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. *J Clin Invest.* 2007 Dec;117(12):3988-4002. PubMed PMID: 18060036.
- [210] Noguera-Troise I, Daly C, Papadopoulos NJ, Coetsee S, Boland P, Gale NW, et al. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Novartis Found Symp.* 2007;283:106-20; discussion 21-5, 238-41. PubMed PMID: 18300417.

- [211] Ridgway J, Zhang G, Wu Y, Stawicki S, Liang WC, Chanthery Y, et al. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature*. 2006 Dec 21;444(7122):1083-7. PubMed PMID: 17183323.
- [212] Pannuti A, Foreman K, Rizzo P, Osipo C, Golde T, Osborne B, et al. Targeting Notch to target cancer stem cells. *Clin Cancer Res*. 2010 Jun 15;16(12):3141-52. PubMed PMID: 20530696. Pubmed Central PMCID: 3008160.
- [213] Foreman K, Rizzo P, Osipo C, and Miele L. . Stem Cells and Cancer: The Cancer Stem Cell Hypothesis; *Cancer Drug Discovery and Development* 2009:pp 3-14.
- [214] Tominaga Y, Wang A, Wang RH, Wang X, Cao L, Deng CX. Genistein inhibits Brca1 mutant tumor growth through activation of DNA damage checkpoints, cell cycle arrest, and mitotic catastrophe. *Cell Death Differ*. 2007 Mar;14(3):472-9. PubMed PMID: 17024228.
- [215] Chen Y, Shu W, Chen W, Wu Q, Liu H, Cui G. Curcumin, both histone deacetylase and p300/CBP-specific inhibitor, represses the activity of nuclear factor kappa B and Notch 1 in Raji cells. *Basic Clin Pharmacol Toxicol*. 2007 Dec;101(6):427-33. PubMed PMID: 17927689.
- [216] Cho OH, Shin HM, Miele L, Golde TE, Fauq A, Minter LM, et al. Notch regulates cytolytic effector function in CD8+ T cells. *J Immunol*. 2009 Mar 15;182(6):3380-9. PubMed PMID: 19265115.
- [217] Jang MS, Miao H, Carlesso N, Shelly L, Zlobin A, Darack N, et al. Notch-1 regulates cell death independently of differentiation in murine erythroleukemia cells through multiple apoptosis and cell cycle pathways. *J Cell Physiol*. 2004 Jun;199(3):418-33. PubMed PMID: 15095289.
- [218] Nickoloff BJ, Qin JZ, Chaturvedi V, Denning MF, Bonish B, Miele L. Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-kappaB and PPARgamma. *Cell Death Differ*. 2002 Aug;9(8):842-55. PubMed PMID: 12107827.
- [219] Wang Y, Chan SL, Miele L, Yao PJ, Mackes J, Ingram DK, et al. Involvement of Notch signaling in hippocampal synaptic plasticity. *Proc Natl Acad Sci U S A*. 2004 Jun 22;101(25):9458-62. PubMed PMID: 15190179.
- [220] Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res*. 2001 Jul-Aug;21(4B):2895-900. PubMed PMID: 11712783.
- [221] Milele LT, N. Ivy, PS. The Cancer Stem Cell Hypothesis, Embryonic Signaling Pathways, and Therapeutic: Targeting an Elusive Concept. 2009.
- [222] Prosperi JR, Goss KH. A Wnt-ow of opportunity: targeting the Wnt/beta-catenin pathway in breast cancer. *Current drug targets*. 2010 Sep;11(9):1074-88. PubMed PMID: 20545611.

- [223] Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. *Science*. 2004 Mar 5;303(5663):1483-7. PubMed PMID: 15001769.
- [224] Akiyama T. Wnt/beta-catenin signaling. *Cytokine Growth Factor Rev*. 2000 Dec;11(4):273-82. PubMed PMID: 10959075.
- [225] Goss KH, Kahn M. Targeting the Wnt pathway in cancer. New York: Springer; 2011.
- [226] van Amerongen R, Mikels A, Nusse R. Alternative wnt signaling is initiated by distinct receptors. *Sci Signal*. 2008;1(35). PubMed PMID: 18765832.
- [227] Polakis P. The many ways of Wnt in cancer. *Curr Opin Genet Dev*. 2007 Feb;17(1):45-51. PubMed PMID: 17208432.
- [228] Polakis P. Wnt signaling and cancer. *Genes Dev*. 2000 Aug 1;14(15):1837-51. PubMed PMID: 10921899.
- [229] Turashvili G, Bouchal J, Burkadze G, Kolar Z. Wnt signaling pathway in mammary gland development and carcinogenesis. *Pathobiology*. 2006;73(5):213-23. PubMed PMID: 17314492.
- [230] Yamaguchi TP. Genetics of Wnt signaling during early mammalian development. *Methods Mol Biol*. 2008;468:287-305. PubMed PMID: 19099264.
- [231] Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell*. 2006 Nov 3;127(3):469-80. PubMed PMID: 17081971.
- [232] He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, et al. Identification of c-MYC as a target of the APC pathway. *Science*. 1998 Sep 4;281(5382):1509-12. PubMed PMID: 9727977.
- [233] Lin SY, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y, et al. Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. *Proc Natl Acad Sci U S A*. 2000 Apr 11;97(8):4262-6. PubMed PMID: 10759547.
- [234] Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, et al. Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *Proc Natl Acad Sci U S A*. 1999 Feb 16;96(4):1603-8. PubMed PMID: 9990071.
- [235] Orsulic S, Huber O, Aberle H, Arnold S, Kemler R. E-cadherin binding prevents beta-catenin nuclear localization and beta-catenin/LEF-1-mediated transactivation. *J Cell Sci*. 1999 Apr;112 ( Pt 8):1237-45. PubMed PMID: 10085258.
- [236] Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature*. 1999 Apr 1;398(6726):422-6. PubMed PMID: 10201372.

- [237] Finch PW, He X, Kelley MJ, Uren A, Schaudies RP, Popescu NC, et al. Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. *Proc Natl Acad Sci U S A*. 1997 Jun 24;94(13):6770-5. PubMed PMID: 9192640.
- [238] Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. *Nat Rev Drug Discov*. 2006 Dec;5(12):997-1014. PubMed PMID: 17139285.
- [239] Bilic J, Huang YL, Davidson G, Zimmermann T, Cruciat CM, Bienz M, et al. Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science*. 2007 Jun 15;316(5831):1619-22. PubMed PMID: 17569865.
- [240] Davidson G, Wu W, Shen J, Bilic J, Fenger U, Stannek P, et al. Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction. *Nature*. 2005 Dec 8;438(7069):867-72. PubMed PMID: 16341016.
- [241] Roose J, Molenaar M, Peterson J, Hurenkamp J, Brantjes H, Moerer P, et al. The Xenopus Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature*. 1998 Oct 8;395(6702):608-12. PubMed PMID: 9783587.
- [242] Zeng X, Tamai K, Doble B, Li S, Huang H, Habas R, et al. A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature*. 2005 Dec 8;438(7069):873-7. PubMed PMID: 16341017.
- [243] Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature*. 1998 Jan 22;391(6665):357-62. PubMed PMID: 9450748.
- [244] Hsieh JC. Specificity of WNT-receptor interactions. *Front Biosci*. 2004 May 1;9:1333-8. PubMed PMID: 14977548.
- [245] Nusse R, van Ooyen A, Cox D, Fung YK, Varmus H. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. *Nature*. 1984 Jan 12-18;307(5947):131-6. PubMed PMID: 6318122.
- [246] Polakis P. Drugging Wnt signalling in cancer. *EMBO J*. 2012 Jun 13;31(12):2737-46. PubMed PMID: 22617421. Pubmed Central PMCID: 3380214.
- [247] Howe EN, Cochrane DR, Cittelly DM, Richer JK. miR-200c Targets a NF-kappaB Up-Regulated TrkB/NTF3 Autocrine Signaling Loop to Enhance Anoikis Sensitivity in Triple Negative Breast Cancer. *PLoS One*. 2012;7(11):e49987. PubMed PMID: 23185507. Pubmed Central PMCID: 3503774.
- [248] Lacroix-Triki M, Geyer FC, Lambros MB, Savage K, Ellis IO, Lee AH, et al. beta-catenin/Wnt signalling pathway in fibromatosis, metaplastic carcinomas and phyllodes tumours of the breast. *Mod Pathol*. 2010 Nov;23(11):1438-48. PubMed PMID: 20693983.
- [249] Lin X, Zhang X, Wang Q, Li J, Zhang P, Zhao M, et al. Perifosine downregulates MDR1 gene expression and reverses multidrug-resistant phenotype by inhibiting

PI3K/Akt/NF-kappaB signaling pathway in a human breast cancer cell line. *Neoplasma*. 2012;59(3):248-56. PubMed PMID: 22329846.

- [250] Lu W, Lin C, King TD, Chen H, Reynolds RC, Li Y. Silibinin inhibits Wnt/beta-catenin signaling by suppressing Wnt co-receptor LRP6 expression in human prostate and breast cancer cells. *Cellular signalling*. 2012 Dec;24(12):2291-6. PubMed PMID: 22820499. Pubmed Central PMCID: 3466371.
- [251] Wu Y, Ginther C, Kim J, Mosher N, Chung S, Slamon D, et al. Expression of Wnt3 Activates Wnt/beta-Catenin Pathway and Promotes EMT-like Phenotype in Trastuzumab-Resistant HER2-Overexpressing Breast Cancer Cells. *Mol Cancer Res*. 2012 Nov 27. PubMed PMID: 23071104.
- [252] Howe LR, Brown AM. Wnt signaling and breast cancer. *Cancer Biol Ther*. 2004 Jan; 3(1):36-41. PubMed PMID: 14739782.
- [253] Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/beta-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. *The American journal of pathology*. 2010 Jun;176(6):2911-20. PubMed PMID: 20395444. Pubmed Central PMCID: 2877852.
- [254] Milovanovic T, Planutis K, Nguyen A, Marsh JL, Lin F, Hope C, et al. Expression of Wnt genes and frizzled 1 and 2 receptors in normal breast epithelium and infiltrating breast carcinoma. *Int J Oncol*. 2004 Nov;25(5):1337-42. PubMed PMID: 15492823.
- [255] Nguyen A, Rosner A, Milovanovic T, Hope C, Planutis K, Saha B, et al. Wnt pathway component LEF1 mediates tumor cell invasion and is expressed in human and murine breast cancers lacking ErbB2 (her-2/neu) overexpression. *Int J Oncol*. 2005 Oct; 27(4):949-56. PubMed PMID: 16142310.
- [256] Xu W, Lin H, Zhang Y, Chen X, Hua B, Hou W, et al. Compound Kushen Injection suppresses human breast cancer stem-like cells by down-regulating the canonical Wnt/beta-catenin pathway. *Journal of experimental & clinical cancer research : CR*. 2011;30:103. PubMed PMID: 22032476. Pubmed Central PMCID: 3219673.
- [257] Bafico A, Liu G, Goldin L, Harris V, Aaronson SA. An autocrine mechanism for constitutive Wnt pathway activation in human cancer cells. *Cancer Cell*. 2004 Nov;6(5): 497-506. PubMed PMID: 15542433.
- [258] Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A*. 2003 Jul 8;100(14):8418-23. PubMed PMID: 12829800.
- [259] Yang L, Wu X, Wang Y, Zhang K, Wu J, Yuan YC, et al. FZD7 has a critical role in cell proliferation in triple negative breast cancer. *Oncogene*. 2011 Oct 27;30(43):4437-46. PubMed PMID: 21532620.
- [260] Matsuda Y, Schlange T, Oakeley EJ, Boulay A, Hynes NE. WNT signaling enhances breast cancer cell motility and blockade of the WNT pathway by sFRP1 suppresses



- MDA-MB-231 xenograft growth. *Breast Cancer Res.* 2009;11(3):R32. PubMed PMID: 19473496. Pubmed Central PMCID: 2716500.
- [261] Chien AJ, Moore EC, Lonsdorf AS, Kulikauskas RM, Rothberg BG, Berger AJ, et al. Activated Wnt/beta-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. *Proc Natl Acad Sci U S A.* 2009 Jan 27;106(4):1193-8. PubMed PMID: 19144919.
- [262] Kawaguchi H. Regulation of osteoarthritis development by Wnt-beta-catenin signaling through the endochondral ossification process. *J Bone Miner Res.* 2009 Jan;24(1):8-11. PubMed PMID: 19016582.
- [263] Khan NI, Bradstock KF, Bendall LJ. Activation of Wnt/beta-catenin pathway mediates growth and survival in B-cell progenitor acute lymphoblastic leukaemia. *Br J Haematol.* 2007 Aug;138(3):338-48. PubMed PMID: 17614820.
- [264] Schulenburg A, Cech P, Herbacek I, Marian B, Wrba F, Valent P, et al. CD44-positive colorectal adenoma cells express the potential stem cell markers musashi antigen (msi1) and ephrin B2 receptor (EphB2). *J Pathol.* 2007 Oct;213(2):152-60. PubMed PMID: 17708598.
- [265] Teng Y, Wang X, Wang Y, Ma D. Wnt/beta-catenin signaling regulates cancer stem cells in lung cancer A549 cells. *Biochem Biophys Res Commun.* 2010 Feb 12;392(3):373-9. PubMed PMID: 20074550.
- [266] Woodward WA, Chen MS, Behbod F, Alfaro MP, Buchholz TA, Rosen JM. WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. *Proc Natl Acad Sci U S A.* 2007 Jan 9;104(2):618-23. PubMed PMID: 17202265.
- [267] Yang W, Yan HX, Chen L, Liu Q, He YQ, Yu LX, et al. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res.* 2008 Jun 1;68(11):4287-95. PubMed PMID: 18519688.
- [268] Ysebaert L, Chicanne G, Demur C, De Toni F, Prade-Houdellier N, Ruidavets JB, et al. Expression of beta-catenin by acute myeloid leukemia cells predicts enhanced clonogenic capacities and poor prognosis. *Leukemia.* 2006 Jul;20(7):1211-6. PubMed PMID: 16688229.
- [269] Li Y, Welm B, Podsypanina K, Huang S, Chamorro M, Zhang X, et al. Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. *Proc Natl Acad Sci U S A.* 2003 Dec 23;100(26):15853-8. PubMed PMID: 14668450.
- [270] Cohen P, Frame S. The renaissance of GSK3. *Nat Rev Mol Cell Biol.* 2001 Oct;2(10):769-76. PubMed PMID: 11584304.
- [271] Pap M, Cooper GM. Role of translation initiation factor 2B in control of cell survival by the phosphatidylinositol 3-kinase/Akt/glycogen synthase kinase 3beta signaling pathway. *Mol Cell Biol.* 2002 Jan;22(2):578-86. PubMed PMID: 11756553.

- [272] Yost C, Torres M, Miller JR, Huang E, Kimelman D, Moon RT. The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* 1996 Jun 15;10(12):1443-54. PubMed PMID: 8666229.
- [273] Geng L, Cuneo KC, Cooper MK, Wang H, Sekhar K, Fu A, et al. Hedgehog signaling in the murine melanoma microenvironment. *Angiogenesis.* 2007;10(4):259-67. PubMed PMID: 17762973.
- [274] Chari NS, McDonnell TJ. The sonic hedgehog signaling network in development and neoplasia. *Adv Anat Pathol.* 2007 Sep;14(5):344-52. PubMed PMID: 17717435.
- [275] Evangelista M, Tian H, de Sauvage FJ. The hedgehog signaling pathway in cancer. *Clin Cancer Res.* 2006 Oct 15;12(20 Pt 1):5924-8. PubMed PMID: 17062662.
- [276] Cohen MM, Jr. The hedgehog signaling network. *Am J Med Genet A.* 2003 Nov 15;123A(1):5-28. PubMed PMID: 14556242.
- [277] Lewis MT, Veltmaat JM. Next stop, the twilight zone: hedgehog network regulation of mammary gland development. *J Mammary Gland Biol Neoplasia.* 2004 Apr;9(2):165-81. PubMed PMID: 15300011.
- [278] Sarkar FH, Li Y, Wang Z, Kong D. The role of nutraceuticals in the regulation of Wnt and Hedgehog signaling in cancer. *Cancer metastasis reviews.* 2010 Sep;29(3):383-94. PubMed PMID: 20711635. Pubmed Central PMCID: 2974632.
- [279] Yang L, Xie G, Fan Q, Xie J. Activation of the hedgehog-signaling pathway in human cancer and the clinical implications. *Oncogene.* 2010 Jan 28;29(4):469-81. PubMed PMID: 19935712.
- [280] Yauch RL, Gould SE, Scales SJ, Tang T, Tian H, Ahn CP, et al. A paracrine requirement for hedgehog signalling in cancer. *Nature.* 2008 Sep 18;455(7211):406-10. PubMed PMID: 18754008.
- [281] Kasper M, Jaks V, Fiaschi M, Toftgard R. Hedgehog signalling in breast cancer. *Carcinogenesis.* 2009 Jun;30(6):903-11. PubMed PMID: 19237605.
- [282] Anton Aparicio LM, Garcia Campelo R, Cassinello Espinosa J, Valladares Ayerbes M, Reboredo Lopez M, Diaz Prado S, et al. Prostate cancer and Hedgehog signalling pathway. *Clin Transl Oncol.* 2007 Jul;9(7):420-8. PubMed PMID: 17652055.
- [283] Vezina CM, Bushman AW. Hedgehog signaling in prostate growth and benign prostate hyperplasia. *Curr Urol Rep.* 2007 Jul;8(4):275-80. PubMed PMID: 18519011.
- [284] Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol.* 2007 Jan 23;17(2):165-72. PubMed PMID: 17196391.
- [285] Medina V, Calvo MB, Diaz-Prado S, Espada J. Hedgehog signalling as a target in cancer stem cells. *Clin Transl Oncol.* 2009 Apr;11(4):199-207. PubMed PMID: 19380296.

- [286] Kalderon D. Similarities between the Hedgehog and Wnt signaling pathways. *Trends Cell Biol.* 2002 Nov;12(11):523-31. PubMed PMID: 12446114.
- [287] Huelsken J, Birchmeier W. New aspects of Wnt signaling pathways in higher vertebrates. *Curr Opin Genet Dev.* 2001 Oct;11(5):547-53. PubMed PMID: 11532397.
- [288] Ingham PW, McMahon AP. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* 2001 Dec 1;15(23):3059-87. PubMed PMID: 11731473.
- [289] Price MA, Kalderon D. Proteolysis of the Hedgehog signaling effector Cubitus interruptus requires phosphorylation by Glycogen Synthase Kinase 3 and Casein Kinase 1. *Cell.* 2002 Mar 22;108(6):823-35. PubMed PMID: 11955435.
- [290] Day TF, Yang Y. Wnt and hedgehog signaling pathways in bone development. *J Bone Joint Surg Am.* 2008 Feb;90 Suppl 1:19-24. PubMed PMID: 18292352.
- [291] Yang SH, Andl T, Grachtchouk V, Wang A, Liu J, Syu LJ, et al. Pathological responses to oncogenic Hedgehog signaling in skin are dependent on canonical Wnt/beta3-catenin signaling. *Nat Genet.* 2008 Sep;40(9):1130-5. PubMed PMID: 19165927.
- [292] Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell.* 2008 Feb 8;132(3):344-62. PubMed PMID: 18267068.
- [293] Brantley DM, Chen CL, Muraoka RS, Bushdid PB, Bradberry JL, Kittrell F, et al. Nuclear factor-kappaB (NF-kappaB) regulates proliferation and branching in mouse mammary epithelium. *Molecular biology of the cell.* 2001 May;12(5):1445-55. PubMed PMID: 11359934. Pubmed Central PMCID: 34596.
- [294] Cao Y, Bonizzi G, Seagroves TN, Greten FR, Johnson R, Schmidt EV, et al. IKKalpha provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. *Cell.* 2001 Dec 14;107(6):763-75. PubMed PMID: 11747812.
- [295] Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, et al. Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. *J Clin Invest.* 1997 Dec 15;100(12):2952-60. PubMed PMID: 9399940.
- [296] Huber MA, Azoitei N, Baumann B, Grunert S, Sommer A, Pehamberger H, et al. NF-kappaB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. *J Clin Invest.* 2004 Aug;114(4):569-81. PubMed PMID: 15314694. Pubmed Central PMCID: 503772.
- [297] Chua HL, Bhat-Nakshatri P, Clare SE, Morimiya A, Badve S, Nakshatri H. NF-kappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2. *Oncogene.* 2007 Feb 1;26(5):711-24. PubMed PMID: 16862183.
- [298] Biswas DK, Shi Q, Bailly S, Strickland I, Ghosh S, Pardee AB, et al. NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apopto-

sis. *Proc Natl Acad Sci U S A*. 2004 Jul 6;101(27):10137-42. PubMed PMID: 15220474. Pubmed Central PMCID: 454178.

- [299] Nakshatri H, Bhat-Nakshatri P, Martin DA, Goulet RJ, Jr., Sledge GW, Jr. Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth. *Mol Cell Biol*. 1997 Jul;17(7):3629-39. PubMed PMID: 9199297. Pubmed Central PMCID: 232215.
- [300] Karin M, Lin A. NF-kappaB at the crossroads of life and death. *Nature immunology*. 2002 Mar;3(3):221-7. PubMed PMID: 11875461.
- [301] Connelly L, Barham W, Onishko HM, Sherrill T, Chodosh LA, Blackwell TS, et al. Inhibition of NF-kappa B activity in mammary epithelium increases tumor latency and decreases tumor burden. *Oncogene*. 2011 Mar 24;30(12):1402-12. PubMed PMID: 21076466.
- [302] Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF-kappaB signaling pathways. *Nature immunology*. 2011 Aug;12(8):695-708. PubMed PMID: 21772278.
- [303] Dejardin E, Bonizzi G, Bellahcene A, Castronovo V, Merville MP, Bours V. Highly-expressed p100/p52 (NFKB2) sequesters other NF-kappa B-related proteins in the cytoplasm of human breast cancer cells. *Oncogene*. 1995 Nov 2;11(9):1835-41. PubMed PMID: 7478612.
- [304] Cogswell PC, Guttridge DC, Funkhouser WK, Baldwin AS, Jr. Selective activation of NF-kappa B subunits in human breast cancer: potential roles for NF-kappa B2/p52 and for Bcl-3. *Oncogene*. 2000 Feb 24;19(9):1123-31. PubMed PMID: 10713699.
- [305] Wang X, Belguise K, Kersual N, Kirsch KH, Mineva ND, Galtier F, et al. Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. *Nature cell biology*. 2007 Apr;9(4):470-8. PubMed PMID: 17369819. Pubmed Central PMCID: 2394707.
- [306] Demicco EG, Kavanagh KT, Romieu-Mourez R, Wang X, Shin SR, Landesman-Bollag E, et al. RelB/p52 NF-kappaB complexes rescue an early delay in mammary gland development in transgenic mice with targeted superrepressor IkappaB-alpha expression and promote carcinogenesis of the mammary gland. *Mol Cell Biol*. 2005 Nov; 25(22):10136-47. PubMed PMID: 16260626. Pubmed Central PMCID: 1280249.
- [307] Connelly L, Robinson-Benion C, Chont M, Saint-Jean L, Li H, Polosukhin VV, et al. A transgenic model reveals important roles for the NF-kappa B alternative pathway (p100/p52) in mammary development and links to tumorigenesis. *J Biol Chem*. 2007 Mar 30;282(13):10028-35. PubMed PMID: 17261585.
- [308] Cao Y, Luo JL, Karin M. IkappaB kinase alpha kinase activity is required for self-renewal of ErbB2/Her2-transformed mammary tumor-initiating cells. *Proc Natl Acad Sci U S A*. 2007 Oct 2;104(40):15852-7. PubMed PMID: 17890319. Pubmed Central PMCID: 2000410.

- [309] Pratt MA, Tibbo E, Robertson SJ, Jansson D, Hurst K, Perez-Iratxeta C, et al. The canonical NF-kappaB pathway is required for formation of luminal mammary neoplasias and is activated in the mammary progenitor population. *Oncogene*. 2009 Jul 30;28(30):2710-22. PubMed PMID: 19483731.
- [310] Visvader JE. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes Dev*. 2009 Nov 15;23(22):2563-77. PubMed PMID: 19933147. Pubmed Central PMCID: 2779757.
- [311] Sasser AK, Sullivan NJ, Studebaker AW, Hendey LF, Axel AE, Hall BM. Interleukin-6 is a potent growth factor for ER-alpha-positive human breast cancer. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2007 Nov;21(13):3763-70. PubMed PMID: 17586727.
- [312] Naugler WE, Karin M. NF-kappaB and cancer-identifying targets and mechanisms. *Curr Opin Genet Dev*. 2008 Feb;18(1):19-26. PubMed PMID: 18440219. Pubmed Central PMCID: 2587362.
- [313] Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell*. 2009 Nov 13;139(4):693-706. PubMed PMID: 19878981. Pubmed Central PMCID: 2783826.
- [314] Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. *Nature*. 2010 Nov 4;468(7320):98-102. PubMed PMID: 20881962. Pubmed Central PMCID: 3084017.
- [315] Jones DH, Nakashima T, Sanchez OH, Kozieradzki I, Komarova SV, Sarosi I, et al. Regulation of cancer cell migration and bone metastasis by RANKL. *Nature*. 2006 Mar 30;440(7084):692-6. PubMed PMID: 16572175.
- [316] Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, et al. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. *Nature*. 2010 Nov 4;468(7320):103-7. PubMed PMID: 20881963.
- [317] Liu M, Sakamaki T, Casimiro MC, Willmarth NE, Quong AA, Ju X, et al. The canonical NF-kappaB pathway governs mammary tumorigenesis in transgenic mice and tumor stem cell expansion. *Cancer Res*. 2010 Dec 15;70(24):10464-73. PubMed PMID: 21159656. Pubmed Central PMCID: 3010731.
- [318] Korkaya H, Paulson A, Iovino F, Wicha MS. HER2 regulates the mammary stem/progenitor cell population driving tumorigenesis and invasion. *Oncogene*. 2008 Oct 16;27(47):6120-30. PubMed PMID: 18591932. Pubmed Central PMCID: 2602947.
- [319] Merkhofer EC, Cogswell P, Baldwin AS. Her2 activates NF-kappaB and induces invasion through the canonical pathway involving IKKalpha. *Oncogene*. 2010 Feb 25;29(8):1238-48. PubMed PMID: 19946332. Pubmed Central PMCID: 2829103.

- [320] Cragg GM, Newman DJ, Yang SS. Natural product extracts of plant and marine origin having antileukemia potential. The NCI experience. *Journal of natural products*. 2006 Mar;69(3):488-98. PubMed PMID: 16562862.
- [321] Miller GT, and S. Spoolman. *Living in the Environment: Principles, Connections, and Solutions*. Brooks-Cole, Belmont, CA. 2011.
- [322] Li Y, Wicha MS, Schwartz SJ, Sun D. Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds. *J Nutr Biochem*. Sep;22(9):799-806. PubMed PMID: 21295962.
- [323] Anderson H, Hills M, Zabaglo L, A'Hern R, Leary AF, Haynes BP, et al. Relationship between estrogen receptor, progesterone receptor, HER-2 and Ki67 expression and efficacy of aromatase inhibitors in advanced breast cancer. *Ann Oncol*. 2011 Feb 1. PubMed PMID: 21285137.
- [324] Chen S, Oh SR, Phung S, Hur G, Ye JJ, Kwok SL, et al. Anti-aromatase activity of phytochemicals in white button mushrooms (*Agaricus bisporus*). *Cancer Res*. 2006 Dec 15;66(24):12026-34. PubMed PMID: 17178902.
- [325] Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Janicke F, et al. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *J Clin Oncol*. 2001 Sep 15;19(18):3808-16. PubMed PMID: 11559718.
- [326] Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N Engl J Med*. 2003 Nov 6;349(19):1793-802. PubMed PMID: 14551341.
- [327] Winer EP, Hudis C, Burstein HJ, Chlebowski RT, Ingle JN, Edge SB, et al. American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for women with hormone receptor-positive breast cancer: status report 2002. *J Clin Oncol*. 2002 Aug 1;20(15):3317-27. PubMed PMID: 12149306.
- [328] Ahmad A, Ali S, Wang Z, Ali AS, Sethi S, Sakr WA, et al. 3, 3'-diindolylmethane enhances taxotere-induced growth inhibition of breast cancer cells through down-regulation of FoxM1. *Int J Cancer*. 2011 Dec 10. PubMed PMID: 21154750.
- [329] Manfredi JJ, Horwitz SB. Taxol: an antimetabolic agent with a new mechanism of action. *Pharmacol Ther*. 1984;25(1):83-125. PubMed PMID: 6149569.
- [330] Li Y, Bhuiyan M, Alhasan S, Senderowicz AM, Sarkar FH. Induction of apoptosis and inhibition of c-erbB-2 in breast cancer cells by flavopiridol. *Clin Cancer Res*. 2000 Jan;6(1):223-9. PubMed PMID: 10656453.
- [331] Somers-Edgar TJ, Scandlyn MJ, Stuart EC, Le Nedelec MJ, Valentine SP, Rosengren RJ. The combination of epigallocatechin gallate and curcumin suppresses ER alpha-

- breast cancer cell growth *in vitro* and *in vivo*. *Int J Cancer*. 2008 May 1;122(9):1966-71. PubMed PMID: 18098290.
- [332] Choi WY, Kim GY, Lee WH, Choi YH. Sanguinarine, a benzophenanthridine alkaloid, induces apoptosis in MDA-MB-231 human breast carcinoma cells through a reactive oxygen species-mediated mitochondrial pathway. *Chemotherapy*. 2008;54(4):279-87. PubMed PMID: 18667818.
- [333] Gu H, Rao S, Zhao J, Wang J, Mu R, Rong J, et al. Gambogic acid reduced bcl-2 expression via p53 in human breast MCF-7 cancer cells. *J Cancer Res Clin Oncol*. 2009 Dec;135(12):1777-82. PubMed PMID: 19582475.
- [334] Jiang J, Grieb B, Thyagarajan A, Sliva D. Ganoderic acids suppress growth and invasive behavior of breast cancer cells by modulating AP-1 and NF-kappaB signaling. *International journal of molecular medicine*. 2008 May;21(5):577-84. PubMed PMID: 18425349.
- [335] Campbell CT, Prince M, Landry GM, Kha V, Kleiner HE. Pro-apoptotic effects of 1'-acetoxychavicol acetate in human breast carcinoma cells. *Toxicol Lett*. 2007 Sep 28;173(3):151-60. PubMed PMID: 17766064.
- [336] Banerjee S, Bueso-Ramos C, Aggarwal BB. Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloprotease 9. *Cancer Res*. 2002 Sep 1;62(17):4945-54. PubMed PMID: 12208745.
- [337] Ahmad A, Wang Z, Ali R, Maitah MY, Kong D, Banerjee S, et al. Apoptosis-inducing effect of garcinol is mediated by NF-kappaB signaling in breast cancer cells. *J Cell Biochem*. 2010 Apr 15;109(6):1134-41. PubMed PMID: 20108249.
- [338] Ahmad A, Banerjee S, Wang Z, Kong D, Sarkar FH. Plumbagin-induced apoptosis of human breast cancer cells is mediated by inactivation of NF-kappaB and Bcl-2. *J Cell Biochem*. 2008 Dec 15;105(6):1461-71. PubMed PMID: 18980240.
- [339] Murillo G, Peng X, Torres KE, Mehta RG. Deguelin inhibits growth of breast cancer cells by modulating the expression of key members of the Wnt signaling pathway. *Cancer Prev Res (Phila)*. 2009 Nov;2(11):942-50. PubMed PMID: 19861542.
- [340] Lee SO, Jeong YJ, Im HG, Kim CH, Chang YC, Lee IS. Silibinin suppresses PMA-induced MMP-9 expression by blocking the AP-1 activation via MAPK signaling pathways in MCF-7 human breast carcinoma cells. *Biochem Biophys Res Commun*. 2007 Mar 2;354(1):165-71. PubMed PMID: 17214970.
- [341] Cornwell T, Cohick W, Raskin I. Dietary phytoestrogens and health. *Phytochemistry*. 2004 Apr;65(8):995-1016. PubMed PMID: 15110680.
- [342] Murphy PA, Song T, Buseman G, Barua K, Beecher GR, Trainer D, et al. Isoflavones in retail and institutional soy foods. *J Agric Food Chem*. 1999 Jul;47(7):2697-704. PubMed PMID: 10552547.

- [343] Amin AR, Kucuk O, Khuri FR, Shin DM. Perspectives for cancer prevention with natural compounds. *J Clin Oncol*. 2009 Jun 1;27(16):2712-25. PubMed PMID: 19414669.
- [344] Sirtori CR, Arnoldi A, Johnson SK. Phytoestrogens: end of a tale? *Ann Med*. 2005;37(6):423-38. PubMed PMID: 16203615.
- [345] Matsumura A, Ghosh A, Pope GS, Darbre PD. Comparative study of oestrogenic properties of eight phytoestrogens in MCF7 human breast cancer cells. *The Journal of steroid biochemistry and molecular biology*. 2005 Apr;94(5):431-43. PubMed PMID: 15876408.
- [346] Casanova M, You L, Gaido KW, Archibeque-Engle S, Janszen DB, Heck HA. Developmental effects of dietary phytoestrogens in Sprague-Dawley rats and interactions of genistein and daidzein with rat estrogen receptors alpha and beta *in vitro*. *Toxicological sciences: an official journal of the Society of Toxicology*. 1999 Oct;51(2):236-44. PubMed PMID: 10543025.
- [347] Chrzan BG, Bradford PG. Phytoestrogens activate estrogen receptor beta1 and estrogenic responses in human breast and bone cancer cell lines. *Mol Nutr Food Res*. 2007 Feb;51(2):171-7. PubMed PMID: 17266178.
- [348] Privat M, Aubel C, Arnould S, Communal Y, Ferrara M, Bignon YJ. Breast cancer cell response to genistein is conditioned by BRCA1 mutations. *Biochem Biophys Res Commun*. 2009 Feb 13;379(3):785-9. PubMed PMID: 19126406.
- [349] Privat M, Aubel C, Arnould S, Communal Y, Ferrara M, Bignon YJ. AKT and p21 WAF1/CIP1 as potential genistein targets in BRCA1-mutant human breast cancer cell lines. *Anticancer Res*. 2010 Jun;30(6):2049-54. PubMed PMID: 20651350.
- [350] Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. *J Biol Chem*. 2002 May 24;277(21):18649-57. PubMed PMID: 11901151.
- [351] Lau TY, Leung LK. Soya isoflavones suppress phorbol 12-myristate 13-acetate-induced COX-2 expression in MCF-7 cells. *Br J Nutr*. 2006 Jul;96(1):169-76. PubMed PMID: 16870006.
- [352] Cappelletti V, Fioravanti L, Miodini P, Di Fronzo G. Genistein blocks breast cancer cells in the G(2)M phase of the cell cycle. *J Cell Biochem*. 2000 Sep 14;79(4):594-600. PubMed PMID: 10996850.
- [353] Choi YH, Zhang L, Lee WH, Park KY. Genistein-induced G2/M arrest is associated with the inhibition of cyclin B1 and the induction of p21 in human breast carcinoma cells. *Int J Oncol*. 1998 Aug;13(2):391-6. PubMed PMID: 9664138.
- [354] Li Z, Li J, Mo B, Hu C, Liu H, Qi H, et al. Genistein induces G2/M cell cycle arrest via stable activation of ERK1/2 pathway in MDA-MB-231 breast cancer cells. *Cell Biol Toxicol*. 2008 Oct;24(5):401-9. PubMed PMID: 18224451.



- [355] Lee WY, Huang SC, Tzeng CC, Chang TL, Hsu KF. Alterations of metastasis-related genes identified using an oligonucleotide microarray of genistein-treated HCC1395 breast cancer cells. *Nutr Cancer*. 2007;58(2):239-46. PubMed PMID: 17640171.
- [356] Bergan R, Kyle E, Nguyen P, Trepel J, Ingui C, Neckers L. Genistein-stimulated adherence of prostate cancer cells is associated with the binding of focal adhesion kinase to beta-1-integrin. *Clinical & experimental metastasis*. 1996 Sep;14(4):389-98. PubMed PMID: 8878413.
- [357] Farina HG, Pomies M, Alonso DF, Gomez DE. Antitumor and antiangiogenic activity of soy isoflavone genistein in mouse models of melanoma and breast cancer. *Oncology reports*. 2006 Oct;16(4):885-91. PubMed PMID: 16969510.
- [358] Vantuyghem SA, Wilson SM, Postenka CO, Al-Katib W, Tuck AB, Chambers AF. Dietary genistein reduces metastasis in a postsurgical orthotopic breast cancer model. *Cancer Res*. 2005 Apr 15;65(8):3396-403. PubMed PMID: 15833874.
- [359] Zhang Y, Zhu G, Gu S, Chen X, Hu H, Weng S. Genistein inhibits osteolytic bone metastasis and enhances bone mineral in nude mice. *Environmental Toxicology and Pharmacology*. 2010; 30:37-44.
- [360] Hauck CR, Hsia DA, Schlaepfer DD. The focal adhesion kinase--a regulator of cell migration and invasion. *IUBMB Life*. 2002 Feb;53(2):115-9. PubMed PMID: 12049193.
- [361] McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, Frame MC. The role of focal-adhesion kinase in cancer - a new therapeutic opportunity. *Nat Rev Cancer*. 2005 Jul;5(7):505-15. PubMed PMID: 16069815.
- [362] Lark AL, Livasy CA, Dressler L, Moore DT, Millikan RC, Geradts J, et al. High focal adhesion kinase expression in invasive breast carcinomas is associated with an aggressive phenotype. *Mod Pathol*. 2005 Oct;18(10):1289-94. PubMed PMID: 15861214.
- [363] Lahlou H, Sanguin-Gendreau V, Zuo D, Cardiff RD, McLean GW, Frame MC, et al. Mammary epithelial-specific disruption of the focal adhesion kinase blocks mammary tumor progression. *Proc Natl Acad Sci USA*. 2007 Dec 18;104(51):20302-7. PubMed PMID: 18056629.
- [364] Xu LH, Yang X, Bradham CA, Brenner DA, Baldwin AS, Jr., Craven RJ, et al. The focal adhesion kinase suppresses transformation-associated, anchorage-independent apoptosis in human breast cancer cells. Involvement of death receptor-related signaling pathways. *J Biol Chem*. 2000 Sep 29;275(39):30597-604. PubMed PMID: 10899173.
- [365] Beviglia L, Golubovskaya V, Xu L, Yang X, Craven RJ, Cance WG. Focal adhesion kinase N-terminus in breast carcinoma cells induces rounding, detachment and apoptosis. *The Biochemical journal*. 2003 Jul 1;373(Pt 1):201-10. PubMed PMID: 12659633.

- [366] Azios NGaD, S. Role of phytoestrogens modulating focal adhesions and focal adhesion kinase (FAK) activity in breast cancer cells. Li J, Li SA, Llombart-Bosch, A, editor. NY: Springer-Verlag; 2005.
- [367] Mitra SK, Lim ST, Chi A, Schlaepfer DD. Intrinsic focal adhesion kinase activity controls orthotopic breast carcinoma metastasis via the regulation of urokinase plasminogen activator expression in a syngeneic tumor model. *Oncogene*. 2006 Jul 27;25(32):4429-40. PubMed PMID: 16547501.
- [368] Wu ZS, Wu Q, Yang JH, Wang HQ, Ding XD, Yang F, et al. Prognostic significance of MMP-9 and TIMP-1 serum and tissue expression in breast cancer. *Int J Cancer*. 2008 May 1;122(9):2050-6. PubMed PMID: 18172859.
- [369] Perentes JY, Kirkpatrick ND, Nagano S, Smith EY, Shaver CM, Sgroi D, et al. Cancer cell associated MT1-MMP promotes blood vessel invasion and distant metastasis in triple-negative mammary tumors. *Cancer Res*. 2011 May 13. PubMed PMID: 21571860.
- [370] Shao ZM, Wu J, Shen ZZ, Barsky SH. Genistein exerts multiple suppressive effects on human breast carcinoma cells. *Cancer Res*. 1998 Nov 1;58(21):4851-7. PubMed PMID: 9809990.
- [371] Shao ZM, Wu J, Shen ZZ, Barsky SH. Genistein inhibits both constitutive and EGF-stimulated invasion in ER-negative human breast carcinoma cell lines. *Anticancer Res*. 1998 May-Jun;18(3A):1435-9. PubMed PMID: 9673352.
- [372] Kousidou OC, Mitropoulou TN, Roussidis AE, Kletsas D, Theocharis AD, Karamanos NK. Genistein suppresses the invasive potential of human breast cancer cells through transcriptional regulation of metalloproteinases and their tissue inhibitors. *Int J Oncol*. 2005 Apr;26(4):1101-9. PubMed PMID: 15754008.
- [373] Silva D. Signaling pathways responsible for cancer cell invasion as targets for cancer therapy. *Current cancer drug targets*. 2004;Jun 4(4):327-36.
- [374] Cao Y, Karin M. NF-kappaB in mammary gland development and breast cancer. *J Mammary Gland Biol Neoplasia*. 2003 Apr;8(2):215-23. PubMed PMID: 14635796.
- [375] Li Y, Ahmed F, Ali S, Philip PA, Kucuk O, Sarkar FH. Inactivation of nuclear factor kappaB by soy isoflavone genistein contributes to increased apoptosis induced by chemotherapeutic agents in human cancer cells. *Cancer Res*. 2005 Aug 1;65(15):6934-42. PubMed PMID: 16061678.
- [376] Li Z, Li J, Mo B, Hu C, Liu H, Qi H, et al. Genistein induces cell apoptosis in MDA-MB-231 breast cancer cells via the mitogen-activated protein kinase pathway. *Toxicol In Vitro*. 2008 Oct;22(7):1749-53. PubMed PMID: 18761399.
- [377] Vanden Berghe W, Dijsselbloem N, Vermeulen L, Ndlovu MN, Boone E, Haegeman G. Attenuation of mitogen- and stress-activated protein kinase-1-driven nuclear fac-

- tor-kappaB gene expression by soy isoflavones does not require estrogenic activity. *Cancer Res.* 2006 May 1;66(9):4852-62. PubMed PMID: 16651441.
- [378] Kazi A, Daniel KG, Smith DM, Kumar NB, Dou QP. Inhibition of the proteasome activity, a novel mechanism associated with the tumor cell apoptosis-inducing ability of genistein. *Biochemical pharmacology.* 2003 Sep 15;66(6):965-76. PubMed PMID: 12963483.
- [379] Sakamoto T, Horiguchi H, Oguma E, Kayama F. Effects of diverse dietary phytoestrogens on cell growth, cell cycle and apoptosis in estrogen-receptor-positive breast cancer cells. *The Journal of nutritional biochemistry.* 2010 Sep;21(9):856-64. PubMed PMID: 19800779.
- [380] Clarke DB, Bailey V, Lloyd AS. Determination of phytoestrogens in dietary supplements by LC-MS/MS. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2008 May;25(5):534-47. PubMed PMID: 18478479.
- [381] Khaodhiar L, Ricciotti HA, Li L, Pan W, Schickel M, Zhou J, et al. Daidzein-rich isoflavone aglycones are potentially effective in reducing hot flashes in menopausal women. *Menopause.* 2008 Jan-Feb;15(1):125-32. PubMed PMID: 18257146.
- [382] Martinez-Montemayor MM, Otero-Franqui E, Martinez J, De La Mota-Peynado A, Cubano LA, Dharmawardhane S. Individual and combined soy isoflavones exert differential effects on metastatic cancer progression. *Clinical & experimental metastasis.* 2010 Oct;27(7):465-80. PubMed PMID: 20517637.
- [383] Ju YH, Allred CD, Allred KF, Karko KL, Doerge DR, Helferich WG. Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice. *J Nutr.* 2001 Nov;131(11):2957-62. PubMed PMID: 11694625.
- [384] Ju YH, Doerge DR, Allred KF, Allred CD, Helferich WG. Dietary genistein negates the inhibitory effect of tamoxifen on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted in athymic mice. *Cancer Res.* 2002 May 1;62(9):2474-7. PubMed PMID: 11980635.
- [385] Ju YH, Fultz J, Allred KF, Doerge DR, Helferich WG. Effects of dietary daidzein and its metabolite, equol, at physiological concentrations on the growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in ovariectomized athymic mice. *Carcinogenesis.* 2006 Apr;27(4):856-63. PubMed PMID: 16399773.
- [386] Messina MJ, Wood CE. Soy isoflavones, estrogen therapy, and breast cancer risk: analysis and commentary. *Nutr J.* 2008;7:17. PubMed PMID: 18522734.
- [387] Guo TL, Chi RP, Hernandez DM, Auttachoat W, Zheng JF. Decreased 7,12-dimethylbenz[a]anthracene-induced carcinogenesis coincides with the induction of antitumor immunities in adult female B6C3F1 mice pretreated with genistein. *Carcinogenesis.* 2007 Dec;28(12):2560-6. PubMed PMID: 17916904.

- [388] Seo HS, Choi HS, Choi HS, Choi YK, Um JY, Choi I, et al. Phytoestrogens induce apoptosis via extrinsic pathway, inhibiting nuclear factor-kappaB signaling in HER2-overexpressing breast cancer cells. *Anticancer Res.* 2011 Oct;31(10):3301-13. PubMed PMID: 21965740.
- [389] Fletcher RJ. Food sources of phyto-oestrogens and their precursors in Europe. *Br J Nutr.* 2003 Jun;89 Suppl 1:S39-43. PubMed PMID: 12725655.
- [390] Munro IC, Harwood M, Hlywka JJ, Stephen AM, Doull J, Flamm WG, et al. Soy isoflavones: a safety review. *Nutr Rev.* 2003 Jan;61(1):1-33. PubMed PMID: 12638461.
- [391] Golbitz P. Traditional soyfoods: processing and products. *J Nutr.* 1995 Mar;125(3 Suppl):570S-2S. PubMed PMID: 7884535.
- [392] Chua R, Anderson K, Chen J, Hu M. Quality, labeling accuracy, and cost comparison of purified soy isoflavonoid products. *J Altern Complement Med.* 2004 Dec;10(6):1053-60. PubMed PMID: 15674001.
- [393] Lockwood GB. The quality of commercially available nutraceutical supplements and food sources. *J Pharm Pharmacol.* 2011 Jan;63(1):3-10. PubMed PMID: 21155809.
- [394] Shu XO, Zheng Y, Cai H, Gu K, Chen Z, Zheng W, et al. Soy food intake and breast cancer survival. *Jama.* 2009 Dec 9;302(22):2437-43. PubMed PMID: 19996398.
- [395] Guha N, Kwan ML, Quesenberry CP, Jr., Weltzien EK, Castillo AL, Caan BJ. Soy isoflavones and risk of cancer recurrence in a cohort of breast cancer survivors: the Life After Cancer Epidemiology study. *Breast Cancer Res Treat.* 2009 Nov;118(2):395-405. PubMed PMID: 19221874.
- [396] Boyapati SM, Shu XO, Ruan ZX, Dai Q, Cai Q, Gao YT, et al. Soyfood intake and breast cancer survival: a followup of the Shanghai Breast Cancer Study. *Breast Cancer Res Treat.* 2005 Jul;92(1):11-7. PubMed PMID: 15980986.
- [397] Dong JY, Qin LQ. Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. *Breast Cancer Res Treat.* 2011 Jan;125(2):315-23. PubMed PMID: 21113655.
- [398] Wu AH, Yu MC, Tseng CC, Pike MC. Epidemiology of soy exposures and breast cancer risk. *Br J Cancer.* 2008 Jan 15;98(1):9-14. PubMed PMID: 18182974.
- [399] Petrakis NL, Barnes S, King EB, Lowenstein J, Wiencke J, Lee MM, et al. Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. *Cancer Epidemiol Biomarkers Prev.* 1996 Oct;5(10):785-94. PubMed PMID: 8896889.
- [400] Allred CD, Ju YH, Allred KF, Chang J, Helferich WG. Dietary genistin stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein. *Carcinogenesis.* 2001 Oct;22(10):1667-73. PubMed PMID: 11577007.

- [401] Sa G, Das T. Anti cancer effects of curcumin: cycle of life and death. *Cell Div.* 2008;3:14. PubMed PMID: 18834508.
- [402] Ramachandran C, Fonseca HB, Jhabvala P, Escalon EA, Melnick SJ. Curcumin inhibits telomerase activity through human telomerase reverse transcriptase in MCF-7 breast cancer cell line. *Cancer Lett.* 2002 Oct 8;184(1):1-6. PubMed PMID: 12104041.
- [403] Holy JM. Curcumin disrupts mitotic spindle structure and induces micronucleation in MCF-7 breast cancer cells. *Mutat Res.* 2002 Jun 27;518(1):71-84. PubMed PMID: 12063069.
- [404] Liu Q, Loo WT, Sze SC, Tong Y. Curcumin inhibits cell proliferation of MDA-MB-231 and BT-483 breast cancer cells mediated by down-regulation of NFkappaB, cyclinD and MMP-1 transcription. *Phytomedicine : international journal of phytotherapy and phytopharmacology.* 2009 Oct;16(10):916-22. PubMed PMID: 19524420.
- [405] Choudhuri T, Pal S, Aggarwal ML, Das T, Sa G. Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction. *FEBS Lett.* 2002 Feb 13;512(1-3):334-40. PubMed PMID: 11852106.
- [406] Aggarwal BB, Shishodia S, Takada Y, Banerjee S, Newman RA, Bueso-Ramos CE, et al. Curcumin suppresses the paclitaxel-induced nuclear factor-kappaB pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. *Clin Cancer Res.* 2005 Oct 15;11(20):7490-8. PubMed PMID: 16243823.
- [407] Bachmeier B, Nerlich AG, Iancu CM, Cilli M, Schleicher E, Vene R, et al. The chemopreventive polyphenol Curcumin prevents hematogenous breast cancer metastases in immunodeficient mice. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology.* 2007;19(1-4):137-52. PubMed PMID: 17310108.
- [408] Boonrao M, Yodkeeree S, Ampasavate C, Anuchapreeda S, Limtrakul P. The inhibitory effect of turmeric curcuminoids on matrix metalloproteinase-3 secretion in human invasive breast carcinoma cells. *Arch Pharm Res.* 2010 Jul;33(7):989-98. PubMed PMID: 20661707.
- [409] Chiu TL, Su CC. Curcumin inhibits proliferation and migration by increasing the Bax to Bcl-2 ratio and decreasing NF-kappaBp65 expression in breast cancer MDA-MB-231 cells. *International journal of molecular medicine.* 2009 Apr;23(4):469-75. PubMed PMID: 19288022.
- [410] Bachmeier BE, Mohrenz IV, Mirisola V, Schleicher E, Romeo F, Hohneke C, et al. Curcumin downregulates the inflammatory cytokines CXCL1 and -2 in breast cancer cells via NFkappaB. *Carcinogenesis.* 2008 Apr;29(4):779-89. PubMed PMID: 17999991.
- [411] Verma SP, Salamone E, Goldin B. Curcumin and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells 68 *Natural Product Discovery – From the Field, to the Lab Bench, to the Medicine*

Cabinet induced by estrogenic pesticides. *Biochem Biophys Res Commun.* 1997 Apr 28;233(3):692-6. PubMed PMID: 9168916.

- [412] Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. *Eur J Cancer.* 2005 Sep;41(13):1955-68. PubMed PMID: 16081279.
- [413] Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, et al. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res.* 2004 Oct 15;10(20):6847-54. PubMed PMID: 15501961.
- [414] Vareed SK, Kakarala M, Ruffin MT, Crowell JA, Normolle DP, Djuric Z, et al. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev.* 2008 Jun;17(6):1411-7. PubMed PMID: 18559556.
- [415] Garcea G, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, et al. Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *Br J Cancer.* 2004 Mar 8;90(5):1011-5. PubMed PMID: 14997198.
- [416] Carroll RE, Benya RV, Turgeon DK, Vareed S, Neuman M, Rodriguez L, et al. Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. *Cancer Prev Res (Phila).* 2011 Mar;4(3):354-64. PubMed PMID: 21372035.
- [417] Bayet-Robert M, Kwiatkowski F, Leheurteur M, Gachon F, Planchat E, Abrial C, et al. Phase I dose escalation trial of docetaxel plus curcumin in patients with advanced and metastatic breast cancer. *Cancer Biol Ther.* Jan;9(1):8-14. PubMed PMID: 19901561.
- [418] Somasundaram S, Edmund NA, Moore DT, Small GW, Shi YY, Orlowski RZ. Dietary curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer. *Cancer Res.* 2002 Jul 1;62(13):3868-75. PubMed PMID: 12097302.
- [419] Kakarala M, Brenner DE, Korkaya H, Cheng C, Tazi K, Ginestier C, et al. Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. *Breast Cancer Res Treat.* 2010 Aug;122(3):777-85. PubMed PMID: 19898931.
- [420] Burgos-Moron E, Calderon-Montano JM, Salvador J, Robles A, Lopez-Lazaro M. The dark side of curcumin. *Int J Cancer.* 2010 Apr 1;126(7):1771-5. PubMed PMID: 19830693.
- [421] Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.* 1998 May;64(4):353-6. PubMed PMID: 9619120.
- [422] Bhardwaj RK, Glaeser H, Becquemont L, Klotz U, Gupta SK, Fromm MF. Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. *J Pharmacol Exp Ther.* 2002 Aug;302(2):645-50. PubMed PMID: 12130727.

- [423] Kurien BT, Scofield RH. Oral administration of heat-solubilized curcumin for potentially increasing curcumin bioavailability in experimental animals. *Int J Cancer*. 2009 Oct 15;125(8):1992-3. PubMed PMID: 19618459.

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