1 Chapter: General Introduction

1.1 Introduction:

Prior to the early 21st century, cancer was primarily conceptualized as a cell-intrinsic disease, with its origins rooted in genetic mutations and epigenetic modifications within malignant cells [1]. This perspective significantly influenced research and therapeutic approaches, emphasizing the characterization and targeting of specific molecular and cellular abnormalities unique to tumor cells [2]. However, this cell-centric view has since evolved, as growing evidence highlights the systemic nature of cancer. Beyond being a localized disease of aberrant cellular growth, cancer is now recognized as a multifaceted systemic disorder involving the progressive dysregulation of various physiological networks [3]. These include immunological responses, metabolic pathways, neuroendocrine signaling, and interactions with the body's microbial communities [4, 5].

The International Agency for Research on Cancer (IARC) recently published updated estimates for the worldwide cancer statistics by globe region for 2022 [6]. Although one in nine men and one in twelve women pass away from cancer, statistics indicate that one in five men and women will get the disease at some point in their lives [7]. Cancer ranks as the second leading cause of death globally, reflecting its significant burden on public health. Projections indicate that by 2050, the global incidence of new cancer cases will exceed 35 million annually, representing a 77% increase compared to the 2022 estimate of 20 million cases, accompanied by approximately 9.7 million deaths [8]. Despite these alarming figures, advancements in cancer care have contributed to improved survival rates, with an estimated 53.5 million individuals alive within five years of a cancer diagnosis as of 2024 [9]. Among cancer diagnoses, female breast cancer has emerged as the most commonly diagnosed type, accounting for an estimated 2.3 million

new cases annually, or 11.7% of all cancers. It is followed closely by lung cancer (11.4%), colorectal cancer (10%), prostate cancer (7.3%), and stomach cancer (5.6%), reflecting the diverse patterns of cancer prevalence worldwide [10]. These statistics underscore the pressing need for continued advancements in prevention, early detection, and treatment strategies to mitigate the growing global cancer burden [11].

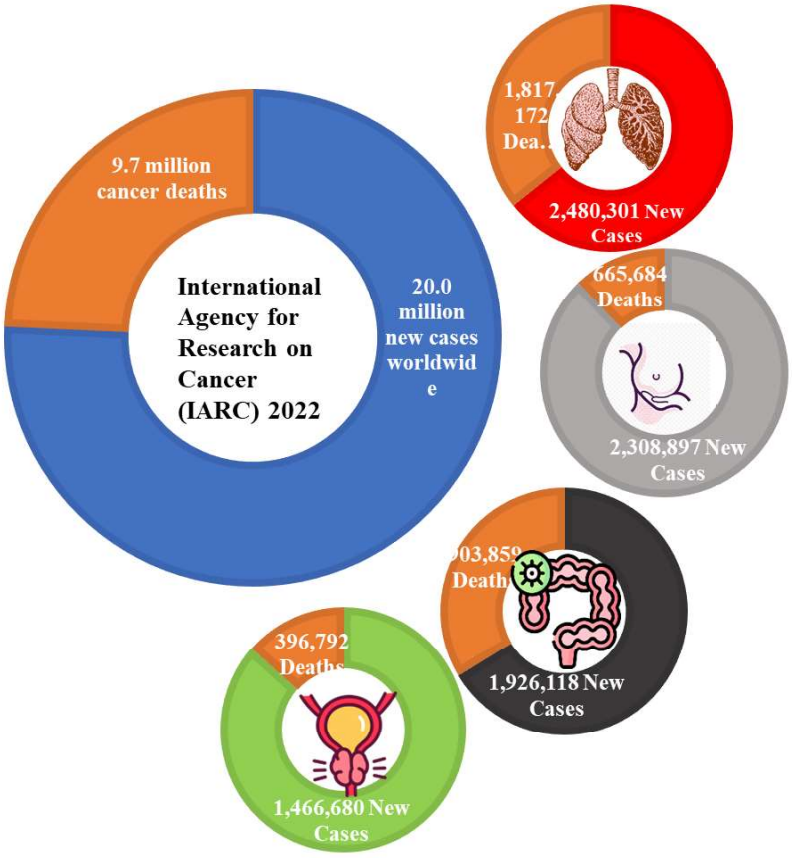

Figure 1: Global cancer incidence and mortality (CA Cancer J Clin. 2024;74:229–263).

Targeted cancer control strategies must be increased globally, as evidenced by the overall prevalence of cancer and the variation in cancer profiles by human development level and worldwide region [12]. Investments in prevention, such as focusing on major

cancer risk factors (such as smoking, being overweight or obese, and infections), can prevent millions of new cancer diagnoses and save countless lives globally, with significant economic and social benefits for nations in the decades to come [13, 14].

1.2 Brief history of breast cancer:

Cancer has been documented in human history for thousands of years, with evidence stretching back to ancient civilizations [15]. The journey from primitive treatments to today's sophisticated therapies reflects humanity's persistent battle against this complex disease. This report explores the historical evolution of cancer understanding and treatment, with special attention to breast cancer as one of the earliest documented forms of the disease [16, 17].

1.2.1 Ancient History and Preliminary Records:

The first recorded mention of human cancer dates back to ancient Egypt. During the Pyramid Age, between 3000 and 2500 BCE, the Edwin Smith Papyrus includes the first documented description of human tumors, including multiple case studies of breast cancer [18].

Physicians in ancient Egypt displayed advanced diagnostic techniques by examining malignancies using both touch and visual means. A number of symptoms are described in their clinical textbooks, such as "swelling with pus spread over the breast, redness, penetration to the bone, inflammation, bulging tumor, and abscess in the breast" [19]. In addition to noting related symptoms like fever, they classified tumors as "hot tumors, cold tumors, oily tumors, solid tumors" [18].

1.2.2 Early Medical Theories and Etymology:

The etymology of the word "cancer" is intriguing. Hippocrates coined the Greek word "karkinos" (crab) to refer to malignant lumps around 400 BCE [20]. This name probably came from the way tumors looked with their long veins, which looked like crab legs. Later, this name was translated to the Latin word "cancer," which also means crab, by the Roman physician Celsus [21].

Early medical beliefs of illness causation shaped our understanding of cancer. Hippocrates put forth the notion of humorism, which attributes disease to abnormalities in the four humors of the body blood, phlegm, yellow bile, and black bile. Galen (129–201 CE), whose works on humoral theory impacted medical practice for decades, expanded on this idea [22]. According to this view, it was crucial for health to maintain balanced humors, and a number of variables, such as occupation, nutrition, and climate, may affect these humors and perhaps cause disease [23].

1.2.3 Historical Overview of the Development of Cancer Knowledge:

Ancient notions continued to have a significant influence on medical knowledge throughout the medieval era and into the Renaissance. A number of theories were put out by doctors to explain cancer, especially breast cancer. Some theories included acidic lymph fluid or milk clots in the mammary ducts [24].

Research on cancer made tremendous strides in the 18th and 19th centuries. In 1775, Percivall Pott made the first definitive connection between environmental exposure and the development of cancer when he discovered a link between chimney sweepers' exposure to soot and squamous cell carcinoma. Understanding environmental carcinogens was made possible by this revolutionary discovery [25].

Rudolph Virchow made the initial link between inflammation and cancer in 1863 when he discovered white blood cells in malignant tissue. Significant advancements in cancer pathology also occurred during this time [26]. By 1838, pathology had moved from being described in detail to being examined under a microscope thanks to Johannes Muller's initial illustrations of the microscopic appearance of cancer [25].

1.2.4 Historic Progress in Diagnosis and Therapy:

Several significant developments in the treatment of cancer occurred in the late 19th century. In 1895, Wilhelm Roentgen made the discovery of X-rays, which led to major advancements in cancer diagnostics [27]. The foundation for radiation therapy in the treatment of cancer was established shortly after Marie and Pierre Curie discovered the radioactive materials polonium and radium in 1898. The first successful use of radiation therapy to treat cancer were being documented in 1899 [28].

1.3 The History of Breast Cancer Treatment

1.3.1 Surgical Approaches

Breast cancer has been recognized as a distinct entity throughout medical history. The treatment of breast cancer reflects the broader evolution of cancer therapy, particularly regarding surgical approaches. For centuries, surgery remained the primary treatment option [29].

In 1882, William Halsted performed the first radical mastectomy for breast cancer a surgical procedure that would remain the standard approach for nearly a century [30]. This aggressive surgery involved removing the entire breast, underlying chest muscles, and axillary lymph nodes, often resulting in significant disfigurement and functional impairment [31].

1.3.2 Modern Therapeutic Evolution

The development of chemotherapy for cancer treatment began accidentally during World War II. In December 1943, a ship carrying nitrogen mustard bombs was bombed, releasing toxic gas that caused bone marrow toxicity and white blood cell death in exposed individuals. Researchers subsequently began investigating these compounds for potential therapeutic applications in cancer, inaugurating the era of systemic cancer treatment [32].

The late 20th and early 21st centuries have seen a revolution in cancer treatment with the development of targeted therapies. Unlike conventional chemotherapy, these molecular targeted agents are designed to specifically interfere with key molecular processes responsible for cancer cell growth and survival [33]. This approach provides opportunities to increase treatment efficacy while potentially reducing side effects compared to traditional cytotoxic therapies [34].

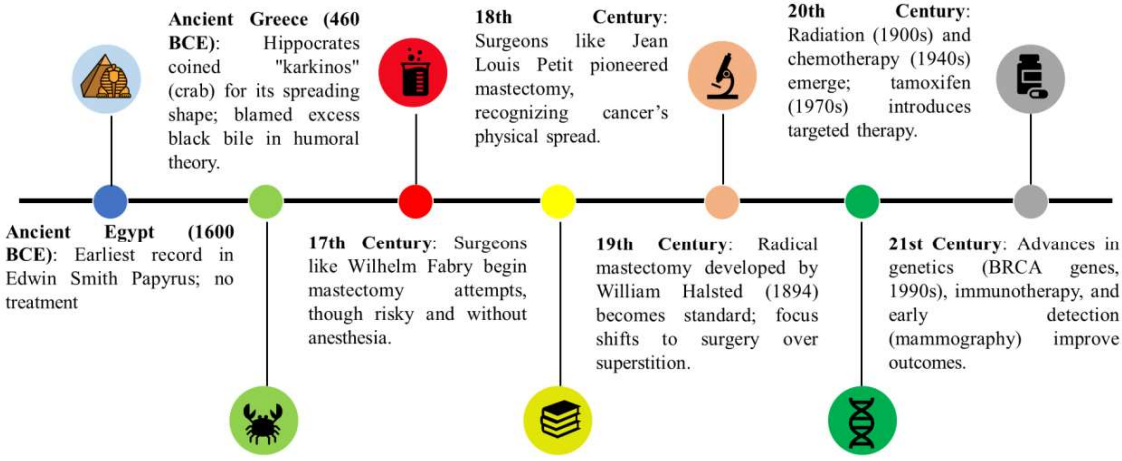

Figure 2: Historic progress in diagnosis and therapy of cancer (Sultan Qaboos Univ Med J. 2014 Apr 7;14(2): e166–e169).

The development of these agents has required new approaches to clinical research, including the identification of biomarkers to select patients most likely to benefit from

specific treatments [35]. As noted in the literature, "These unique challenges include the need to select appropriate pharmacodynamic markers to guide dose and schedule and to identify biomarkers that enable selection of patient populations that are most likely to benefit from the treatment" [29].

1.4 Development of breast cancer:

Breast cancer's origin is not entirely known. Breast cancer development can be influenced by a number of interconnected elements, including hormones, the environment, genetics, sociobiology, and physiology. Numerous illnesses referred to as risk factors have been found to either stimulate or predispose people to cancer [36]. Several risk factors for breast cancer have been found, including: Environmental factors, Sociobiological factors, Genetic factor, A woman's hormonal history, Family risk factors and the immune system [37].

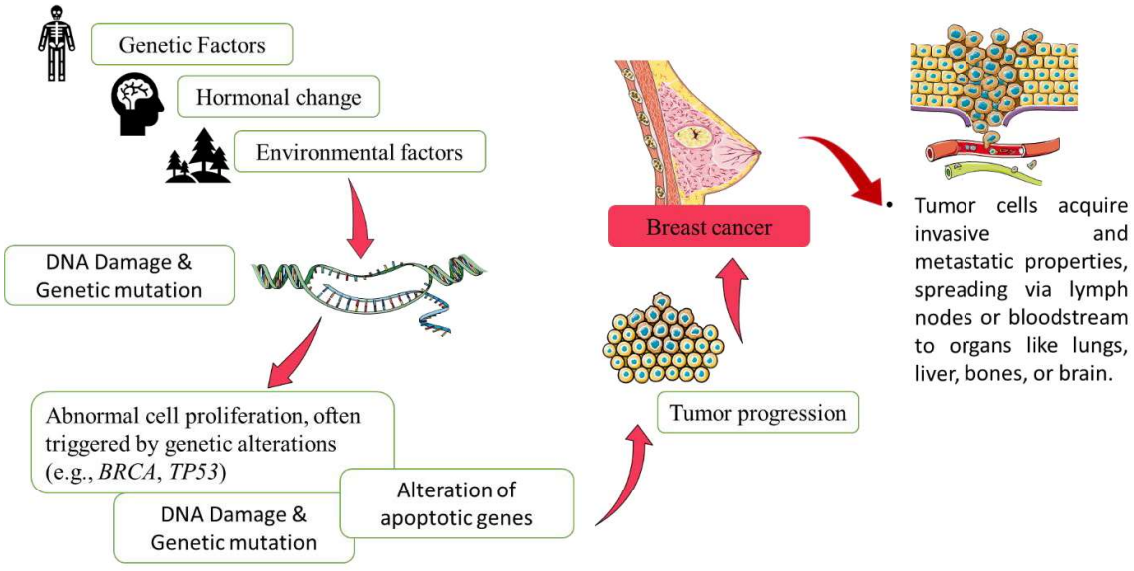

Figure 3: Pathophysiology of breast cancer (Sig Transduct Target Ther **10**, 49 2025 10.1038/s41392-024-02108-4).

1.5 Sirtuins: NAD^+ Dependent Deacylases - Structure, Function, and Cellular Roles:

Sirtuins are a family of NAD+-dependent enzymes that have become important modulators of several cellular functions with important health and disease consequences. There is a protein called class III histone deacetylases (HDACs) that is related to Sir2, which was first found in yeast [38]. They have many biological roles that go beyond their original identification as chromatin modification [39].

1.5.1 Evolutionary Conservation and Structural Organization

Sirtuins are highly conserved proteins that share a common catalytic domain while exhibiting unique structural features that contribute to their diverse cellular functions [40].

All sirtuins are distinguished by their highly conserved catalytic core domain, which is made up of about 275 amino acid residues[40]. This core serves as this protein family's functional hallmark and is necessary for their NAD^+-dependent enzymatic activity. The catalytic domain has two separate subdomains and a distinctive architecture [41].

Large Rossmann-fold Domain: This domain serves as the NAD^+ binding site due to its reverse Rossmann fold structure. A six-stranded parallel β-sheet (β1-3 and β7-9) encircled by eight α-helices (αA, αB, αG, αH, and αJ-M) makes up this domain in SIRT1 [42].

Smaller Zinc-binding Domain: This domain is made up of a flexible helical subdomain and a zinc ribbon [43]. The four conserved cysteine residues in the zinc-binding motif work together to coordinate a zinc ion, creating a zinc-tetrathiolate

structure that is essential for preserving the structural integrity and catalytic activity of the protein [44].

The hydrophobic tunnel created by the gap connecting these two domains is where substrate binding and catalysis take place. For the acyl-lysine substrate and NAD^+ cofactor, the configuration produces unique binding pockets [45].

1.5.2 Subcellular Distribution and Isoform-Specific Functions:

The seven mammalian sirtuin isoforms (SIRT1-7) are distributed across different cellular compartments, enabling compartment-specific regulation of diverse cellular processes [46].

1.5.2.1 Nuclear Sirtuins

Several sirtuin family members primarily localize to the nucleus where they regulate chromatin structure and nuclear processes [40].

SIRT1: Primarily nuclear, SIRT1 deacetylates histones (particularly H3K9 and H4K16) and various transcription factors including p53, NF-κB, and FOXO proteins. It plays crucial roles in DNA repair, gene transcription, metabolism, stress responses, and inflammation [47].

SIRT6: Tightly bound to chromatin, SIRT6 deacetylates H3K9 and H3K56, regulating DNA repair, telomere maintenance, and glucose metabolism. SIRT6 deficiency leads to severe metabolic imbalance, hypoglycemia, and premature death in mice, highlighting its essential role in development and metabolism [48].

SIRT7: Localized primarily in the nucleolus, SIRT7 functions as a histone desuccinylase that plays roles in DNA damage response and chromatin compaction. It is

recruited to DNA double-strand breaks in a PARP1-dependent manner and catalyzes H3K122 desuccinylation, promoting chromatin condensation and DNA repair [49].

1.5.2.2 Mitochondrial Sirtuins

Three sirtuin family members (SIRT3-5) are localized to mitochondria, where they regulate various aspects of mitochondrial function [50].

SIRT3: The most studied mitochondrial sirtuin, SIRT3 deacetylates numerous mitochondrial proteins involved in oxidative phosphorylation, fatty acid oxidation, ketone body production, and antioxidant defense. Deletion of SIRT3 in mice leads to striking mitochondrial protein hyperacetylation and metabolic abnormalities [51].

SIRT4: Possesses ADP-ribosyltransferase activity and can ADP-ribosylate and repress glutamate dehydrogenase (GDH). It also interacts with CRAF and suppresses CRAF-MAPK signaling [52].

SIRT5: Regulates various post-translational lysine modifications beyond acetylation, including malonylation, succinylation, and glutarylation. SIRT5 deacetylates and activates carbamoyl phosphate synthetase 1 (CPS1) and promotes cell survival and proliferation [52].

The distribution of sirtuins across different cellular compartments allows for coordinated regulation of various cellular processes in response to metabolic signals and stress conditions [51].

Research on sirtuins is still being conducted in an effort to clarify their exact mechanisms of action, physiological roles, and potential as therapeutic targets. As we

learn more about these amazing enzymes, we may be able to use their activity to create new ways to prevent age-related illnesses and encourage healthy aging [53].

1.5.3 Dual Nature of Sirtuins in Breast Cancer

1.5.3.1 Tumor-Promoting Functions

Multiple studies have demonstrated that several sirtuins exhibit oncogenic properties in breast cancer contexts [54]. SIRT1, the most extensively studied sirtuin, is significantly upregulated in breast cancer tissues and cells compared to normal breast tissue, with its expression correlating with histological grade, tumor size, and lymph node metastasis [55]. Overexpression of SIRT1 has been shown to significantly promote breast cancer growth both in vitro and in vivo, while knockdown of SIRT1 inhibits these cancer-promoting phenotypes [56].

The tumor-promoting mechanisms of sirtuins include direct interaction with oncogenic pathways [57]. SIRT1 directly interacts with Akt, promoting its activity in breast cancer cells in vitro and correlating positively with expression of Akt and phosphorylated Akt in breast cancer tissues2. This interaction enhances tumor cell proliferation and survival [58].

Metabolic reprogramming: SIRT5 desuccinylates and stabilizes the mitochondrial enzyme glutaminase (GLS), thereby promoting breast cancer tumorigenesis through altered glutamine metabolism [59]. Additionally, SIRT5 reduces reactive oxygen species (ROS) generation while increasing NADPH and GSH levels, creating a favorable environment for tumor progression and metastasis [60].

Resistance to oxidative stress: SIRT6 deacetylates and activates nicotinamide phosphoribosyltransferase (NAMPT) and glucose-6-phosphate dehydrogenase,

increasing NADH levels and promoting breast cancer survival and resistance to oxidative stress [61].

Tamoxifen resistance: SIRT3 expression has been found to be higher in tamoxifen-resistant breast cancer cells, with knockdown of SIRT3 increasing the sensitivity of resistant cells and inducing apoptosis by increasing mitochondrial ROS [62].

1.5.4 Therapeutic Implications and Future Directions:

1.5.4.1 Sirtuin Modulators as Potential Therapeutic Agents

The complex roles of sirtuins in breast cancer suggest potential therapeutic applications for sirtuin modulators:

1. SIRT1 inhibitors: Compounds such as sirtinol have been shown to activate p53 and increase p21 protein levels in SK-BR-3 breast cancer cells, suggesting a potential therapeutic approach for certain breast cancer subtypes [39].

2. SIRT3 modulators: Given SIRT3's context-dependent role in different types of cancer, various SIRT3 activators and inhibitors have been developed to regulate cancer growth. The elucidation of SIRT3's crystal structure has expedited the development of small molecular modulators [63].

3. SIRT5 inhibitors: Targeting SIRT5 with inhibitors has been shown to significantly suppress tumor growth in mouse models without notable toxicity in normal cells. This suggests SIRT5's dispensability in normal conditions but essential role in cancer, highlighting its promise as a therapeutic target [64].

4. Novel compounds: Salermide, a reverse amide compound, exhibits potent inhibitory effects against SIRT1 and SIRT2 in vitro and can induce p53-mediated

apoptosis in breast cancer cells without affecting normal cells, underscoring its therapeutic potential [62].

As research continues to elucidate the mechanistic roles of sirtuins in breast cancer, the potential for developing more targeted and effective therapeutic approaches grows [65]. The integration of sirtuin-targeted treatments with conventional therapies or microRNA-based approaches may offer novel strategies for combating breast cancer, particularly aggressive subtypes with limited treatment options [66].

Future research should focus on further clarifying the context-dependent roles of sirtuins in different breast cancer subtypes and stages, as well as developing more specific and potent sirtuin modulators with improved pharmacokinetic properties and reduced off-target effects. Such advances could significantly impact the landscape of breast cancer treatment in the coming years [67].

1.6 Phytoestrogens and Breast Cancer:

Phytoestrogens, plant-derived compounds with structural similarities to estrogen, have emerged as intriguing factors in breast cancer research [68]. Their ability to interact with estrogen receptors and influence various cellular processes has generated considerable interest in their potential protective or harmful effects on breast cancer risk [69]. This report examines the current understanding of the relationship between phytoestrogens and breast cancer, exploring mechanisms of action, epidemiological evidence, and clinical implications [70].

1.6.1 Classification and Dietary Sources:

Phytoestrogens are plant compounds that exhibit estrogen-like cellular actions due to their structural similarity to 17β-estradiol, the primary female hormone [71]. These compounds are widely distributed in plant foods and are classified into several categories:

1. **Isoflavones**: Primarily found in soy products, isoflavones include genistein and daidzein, which are the most extensively studied phytoestrogens. Soy foods such as tofu, soy milk, and edamame are particularly rich sources of these compounds [72].

2. **Lignans**: These phytoestrogens are present in whole grains, dried beans, peas, fruits, and cruciferous vegetables like broccoli and cauliflower. Flaxseeds are particularly high in lignans, which are metabolized by gut bacteria to form enterolactone and enterodiol [73].

3. **Coumestans**: Found in clover and alfalfa sprouts, coumestans represent another class of phytoestrogens with biological activity [74].

The consumption of these compounds varies significantly across populations, with Asian countries traditionally consuming higher amounts of isoflavones through soy-based diets compared to Western populations [75]. This dietary pattern has been proposed as one explanation for the lower breast cancer rates observed in Asian countries compared to Western nations [76].

1.6.2 Mechanisms of Action in Breast Cancer:

Phytoestrogens exert their biological effects through multiple mechanisms, with their interaction with estrogen receptors (ERs) being the most well-characterized:

1. **Selective ER binding**: Phytoestrogens can bind to both ERα and ERβ, but many demonstrate preferential affinity for ERβ, which appears to be associated with antiproliferative and anticarcinogenic effects [77]. This selective binding is significant because ERα typically mediates the growth-promoting effects of estrogen, while ERβ can counteract these effects [78].

2. **Biphasic effects**: At low concentrations, phytoestrogens may stimulate ER-positive breast cancer cell growth, while at higher concentrations, they can inhibit growth and induce apoptosis (programmed cell death). This dose-dependent behavior may explain some of the conflicting results observed in research [79].

3. **Inhibition of steroidogenic enzymes**: Phytoestrogens can reduce the local production of estrogens in breast tissue by inhibiting key enzymes involved in converting androgens and estrogen sulfate into active estradiol [80]. This mechanism may be particularly relevant in postmenopausal women, where most estrogen is produced in peripheral tissues rather than ovaries [81].

4. **Non-Hormonal Mechanisms:**

Beyond their interactions with estrogen receptors, phytoestrogens influence breast cancer through several non-hormonal pathways:

1. **Cell cycle regulation**: Phytoestrogens have been shown to inhibit cyclin D1 expression while increasing the expression of cyclin-dependent kinase inhibitors (p21 and p27) and the tumor suppressor gene p53, although these effects are typically observed at high concentrations [82, 83].

2. **Anti-angiogenic and anti-inflammatory effects**: Genistein, for example, demonstrates anti-angiogenic and anti-inflammatory properties through

regulation of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 expression [84].

3. **Epigenetic modifications**: Phytoestrogens can induce epigenetic changes that influence gene expression in breast cancer cells [85]. These modifications include alterations in DNA methylation, histone modifications, and expression of non-coding RNAs, which collectively can affect cancer-related gene expression [76].

The mechanisms underlying these effects include selective estrogen receptor modulation, inhibition of estrogen synthesis, cell cycle regulation, anti-inflammatory actions, and epigenetic modifications [86]. However, the biphasic nature of phytoestrogen activity potentially stimulating growth at low concentrations while inhibiting it at higher doses underscores the complexity of their role in breast cancer biology [87].

While dietary consumption of phytoestrogen-rich foods appears generally safe and potentially beneficial, particularly for postmenopausal women, concerns remain regarding high-dose supplements, especially for women with or at high risk for ER-positive breast cancer [88]. As research continues to evolve, a better understanding of how individual factors influence responses to phytoestrogens may enable more personalized and effective approaches to breast cancer prevention and management [89].

1.7 Coumestrol:

Coumestrol, a type of coumestans, basically tetracyclic heterocycles that share a C=C bond and are composed of a fused coumarin and benzofuran ring [90]. Numerous oxygenated coumarins have been identified and are present in young, sprouting legumes

like clover and alfalfa sprouts, making it a common component in various animal feeds [91].

Coumestrol can be also present in chickpeas and alfalfa, which are well known for their estrogenic activity. Coumestrol can enhance ERβ expression, triggering the ERβ signaling pathway in the gut to promote apoptosis [75]. This process has been shown to decrease the number of small intestinal mucosal tumors and colon tumors in $ApcMin^{/+}$ mice [15]. Additionally, coumestrol can influence the activity of the ERβ variant, ERβ2. Wedelolactone, which has a structure similar to coumestrol but with an additional hydroxyl and methoxyl group, functions as an agonist for both ERα and Erβ [92]. It promotes the proliferation of ER-positive cells and enhances the expression of estrogen-responsive genes via the ER genomic signaling pathway [93]. In addition, it activates a rapid, non-genomic estrogen signaling pathway. These effects, however, can be suppressed by the pure ER antagonist ICI 182,780, further supporting wedelolactone's positive role in ER signaling [94].

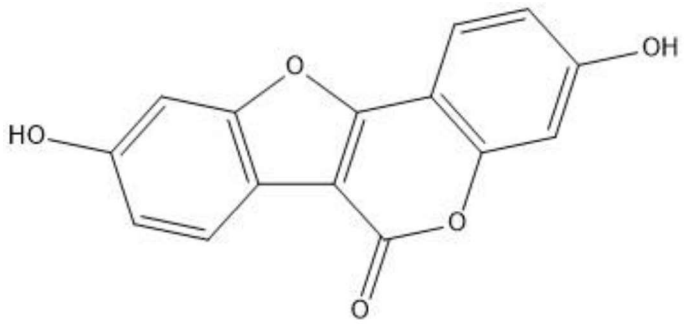

Figure 4: Chemical structure of coumestrol (3,9-dihydroxy-[1]benzofuro[3,2-c]chromen-6-one).

The promising data on phytoestrogens highlight the importance of dietary factors in cancer prevention and suggest that incorporating moderate amounts of phytoestrogen-

rich foods into a balanced diet may contribute to reducing breast cancer risk, particularly when such dietary patterns are established early in life and maintained throughout adulthood [95].

1.8 Aims:

To investigate novel therapeutic strategies targeting sirtuin inhibition and metabolic pathways in breast cancer using phytoestrogens, and 2-(diarylalkyl)aminobenzothiazole derivatives. Specifically, the aims were to:

1) To identify and characterize phytoestrogens as potential sirtuin inhibitors and evaluate their anti-cancer efficacy against breast cancer cells (Chapter 2).

2) To perform a comparative metabolomic analysis of MCF-7 and MDAMB-231 breast cancer cell lines treated with doxorubicin and/or coumestrol using a GC-MS-based approach (Chapter 3).

3) To assess the ability of 2-(diarylalkyl)aminobenzothiazole derivatives to induce autophagy and apoptotic cell death in MCF-7 cells through sirtuin inhibition and p53 activation (Chapter 4).