Breast Cancer

CURRENT TRENDS IN MOLECULAR RESEARCH

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Bentham Books

Breast Cancer: Current Trends in Molecular Research

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FOREWORD

Breast cancer malignancy is now becoming a global leading cause of cancer related death among females all around the world. The current developments in breast cancer research have wrought to increase the life expectancy in patients in the 21st century but a long way to go to cure this deadly disease. Among the major challenges, the heterogeneity in cancer cells make the disease more complex. Researchers have made a significant advancement in studying the heterogeneous features in breast cancer and multiple subsets of breast cancer are discovered. In the molecular biology studies, breast cancer stem-like cells, driver mutations and changes in tumor microenvironment are investigated as potentially hallmarks for the disease progression. Immunological aspects in breast cancer are considered cutting-edge science in recent discoveries. I am happy to look at this book that has incorporated all current advances in the breast cancer research. I congratulate Drs. Suman and Priya for editing a nice compilation of the different pieces of findings of breast cancer research in this book. I hope this will help to understand the recent advances in breast cancer to the researchers' particularly new investigators and clinicians.

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PREFACE

Breast cancer is the most frequent malignancy in women worldwide. It is associated with several risk factors including mutations, inheritance, and environmental factors. The heterogeneity in breast cancer cells shows the complexity of this disease. Despite newer day-to-day developments in cancer research, advanced breast cancer is still challenging to manage. The scientific innovation in breast cancer research has led to an increase in patient's overall survival in the last few decades. For example- the discovery of sensitive screening tools enhanced early-stage detection of breast cancer and further novel treatment regimens improved therapeutic benefits. The current research is also focused on developing new surgical modalities that are minimally invasive, and new radiation modalities with minimal side effects. This book is intended to target a broad audience who has an interest in breast cancer research and therapy. We have incorporated chapters delineating the recent studies on breast cancer with an emphasis on etiology, diagnosis, and therapy. Chapters uncover the new updated information on breast cancer signaling, immune-response, DNA damage, and epigenetic modifications with bioinformatics resources. The book will allow readers to review the data of numerous studies on immunotherapy and gene therapy as well. The latest concept on the use of nanotechnology in breast cancer, has also been reviewed in detail as nanotechnology has opened a paradigm shift in targeted drug delivery in breast cancer. The specific chapter also shows the importance of dietary polyphenol and its role in breast cancer. The application of radiotherapy in breast cancer is also well described, which will be helpful to gain the concept of clinical radiotherapy. In overall, chapters are organized to give the readers a practical and efficient way to familiarize themselves with the newest ongoing research in the field of breast cancer.

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CHAPTER 1

Cellular and Molecular Mechanisms of Breast Cancer Progression

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Abstract: Breast cancer is a common death-related cancer in women globally. Early and non-metastatic stage breast cancers are curable in 70-80% of the patients, while advanced-stage distant organ metastatic breast cancers are incurable with present treatment options. Although multiple risk factors are associated with breast cancer, among them, genetic predispositions in BRCA1 and BRCA2 genes are the most causative factor for breast cancer malignancy. The initiation and progression of breast cancer is a multi-step process, which can initiate either in ducts or lobules of the breast tissues. As time progresses pre-invasive lesions form of breast neoplasm transforms into atypical ductal hyperplasia (ADH), ductal carcinoma *in situ* (DCIS)/lobular carcinoma *in situ* (LCIS), and eventually become invasive carcinoma. The molecular mechanisms behind the initiation and progression of breast cancer are not completely understood. However, epithelial-mesenchymal transition (EMT) is the assurance of malignancy which disrupts endothelial integrity and therefore, it increases the spreading of cancer cells and facilitates metastasis. After the epithelial-mesenchymal transition of tumor cells, tumor cells invade and migrate the neighboring as well as distant tissues, cross the endothelial barrier and enter the blood, and attach to a secondary site, forming metastases. In this chapter, we have reviewed an overview of the molecular mechanisms of breast cancer progression.

Keywords: EMT, Hyperplasia, Malignancy, Treg, Tumor cells.

INTRODUCTION

Breast cancer is the most common carcinoma and has a high incidence rate amongst all types of women cancer worldwide [1]. Breast cancer is a heterogeneous disease, which can be divided into several subtypes based on histological appearance and the expression of molecular markers.

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In the United States, it is approximated that one in eight women will develop breast cancer in her lifetime. This development of breast cancer is a complex process, which is multistep events from initiation, progression, to metastasis. The mechanistic basis of breast cancer metastasis is the epithelial to mesenchymal transition (EMT) [2].

Cancer cell migratory nature is defined by EMT and its converse MET (mesenchymal-epithelial transition) process. There are a series of events that happen when cells transform from epithelial to mesenchymal stages, like cell junction loss and acquiring the migrant nature. During the transformation process, the cells lose their "epithelial" characteristics such as cell to cell adhesion, cell junctions, and cells that express vimentin as the key intermediate filament protein. Undoubtedly, EMT is a shorthand that means changes in the cell shape from coherent "epithelial" monolayer to a migratory fibroblastic or "mesenchymal" phenotype.

The discovery of more driver genes and the complex molecular pathways of breast cancer pave a better understanding of disease progression. Based on the expression profiles of various genes, breast cancer has been categorized into five distinct subtypes: luminal A, luminal B, normal-like, basal-like, and human epithelial growth factor receptor 2 (HER2) types. All these subtypes have different clinical consequences and therapeutic options [3]. The oncogenes strongly influence the changes in malignancy and distant metastasis. Normal breast stromal cells transform into cancer cells by gain-in-function mutations (oncogene). These mutant genes are driver oncogenes that dysregulate apoptotic pathways to become resistance phenotypes. Further constant oncogenic pressure dilutes the effect of existing chemotherapies and thereby leads to poor patient survival. Thus, targeting oncogenic drivers and their downstream signaling molecules are being pursued rationally for breast cancer. For example, luminal or HER2-positive subtypes of breast cancers are getting treated with endocrine therapies or HER2 targeted therapies. In addition, molecular mechanism-based therapies have brought a paradigm shift in recent therapy. Such therapies consist of DNA repair PARP protein inhibitors for BRCA-mutant basal cancer subtype or CDK4/6 inhibitors for advanced ER+ HER2- breast cancers. Several clinical trials are uncovering the potential therapeutic use of immune checkpoint inhibitors as monotherapy or with other target-based therapies for breast cancer. Additionally, the role of different immune cells and molecular markers in the development and metastasis of breast cancer has been explored in past decades which led to the development and use of immunotherapy for breast cancer patients.

The family history of having breast cancer or if any close relatives, such as a mother, sister, or daughter ever been diagnosed with breast cancer, the probability

of having breast cancer rises at an early stage *i.e*., premenopausal age [4 - 6]. Despite the advancements in treatment, the five-year survival risks for patients with metastatic breast cancer are still only very low (22%) [7], and breast cancer still directly affects one in every eight U.S. women [8]. Breast cancers can be sub-categorized based on the expression of distinct molecular and histological markers. Infiltrating ductal carcinoma (~85%) and infiltrating lobular carcinoma (~15%) are categorized as the main histological subtypes of invasive breast cancer [6]. Approximately 75% of all Breast cancer patients have molecular signatures that are designated as hormone receptor-positive cancers, expressing either estrogen receptor (ER) or progesterone receptor (PR) at higher than 1%. Because of the receptor's overexpression, these cancers can be targeted by drugs that directly target the receptors like tamoxifen or aromatase inhibitors. Another group of treatable breast cancers includes human epidermal growth factor receptor 2 (HER2) positive (15-20%), which tend to grow faster than HER2 negative breast cancers, but it can be targeted with anti-Her2 therapies such as trastuzumab [6]. While there has been a success in finding drugs that treat and cure early-stage, ER, PR, and HER2 positive breast cancers, there are no approved target-based therapies to date for triple-negative breast cancer (TNBC). The TNBC patients (~15%) do not display high amounts of any of these molecular markers [6]. For this reason, patients with TNBC have a higher likelihood of recurrence and lower five-year survival rates than those diagnosed with other subtypes of breast cancer.

TYPES OF BREAST CANCER

Breast cancers are classified based on their presence in different areas of the breast such as lobules, ducts, or within tissues. However, based on cell origin, breast cancers are broadly categorized as carcinomas and sarcomas. Carcinomas are the type of breast cancer that arises from epithelial components lying between lobules and terminal ducts. Sarcomas are a very rare form of breast cancer (it is less than 1% of total breast cancer) that arises from the stromal constituents of the breast, these include myofibroblasts and blood vessel cells. These categorizations are inadequate because sometimes a single mammary tumor can be a mixture of different cell types [9 - 11]. Breast cancers generally fall into two subtypes, histological subtypes, and molecular subtypes.

Histological Subtypes

Most breast cancers are diagnosed with carcinoma. Under carcinomas, many different types of breast cancer are recognized based on invasiveness compared to the primary tumor sites. Breast cancers fall under three major groups based on pathological characteristics and invasive properties which are non-invasive (or *in situ*), invasive, and metastatic breast cancer types [9 - 11].

CHAPTER 2

Immune-Endocrine Perspectives of Breast Cancer

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Abstract: Cancer is the consequence of the recalcitrant multiplication of the transformed cells. Cancer cells grow and proliferate at a fast pace and do not follow normal regulation of cell division. Breast cancer is a heterogeneous group of diseases, which is the second leading cause of death among women. Although androgen is primarily considered a male steroid hormone, it also has an important role in the female reproductive system. The literature evidence suggests the role of androgen receptors (AR) in the normal development of the breast. At puberty, the expression of AR is even more than ER, suggesting its importance during the process of sexual development; its activity maintains the ER-induced cell proliferation and normal development of the breast. Epidemiological studies have suggested a positive correlation between high endogenous androgens and the risk of breast cancer in both pre- and postmenopausal women. In both ER and PR-positive breast cancers, AR is expressed in 60-70% of the cases. AR is also reported to be co-expressed with ER in around 80-90% of breast cancer cases and is considered an independent prognostic factor of ER-positive breast cancers. Tumor-microenvironment has a complex role in tumor initiation, progression, and metastasis. Tumor-infiltrating and resident cells secretes a variety of inflammatory and anti-inflammatory cytokines, which in turn either inhibit or promote tumor growth. Immunosuppressive and immuno-inductive effects of androgen have been reported in various studies. Androgens have been reported to influence the adaptive immune system more than the innate immune system in many ways. Crosstalk of androgen and cytokine signaling has many effects in breast cancer epidemiology. So, in this chapter, we will discuss the various immune-endocrine perspectives of breast cancers.

Keywords: Adaptive immunity, Androgens, Apocrine breast cancer, Cancer immunoediting, Dihydrotestosterone, Innate immunity, T cytotoxic cells, T helper cells.

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INTRODUCTION

Cancer is the consequence of the recalcitrant multiplication of the transformed cells of a particular tissue or organ of the body.

Cancer cells grow and proliferate in an uncontrolled manner and do not follow normal constraints on division and are able to invade and colonize the surrounding tissues *via* the process of metastasis. Normal cells abide by specific pro- and anti-signals for processes like proliferation, growth, and differentiation. Development of cancer requires promoting assaults like mutagenesis [1], inflammation [2], and gradual accumulation of genetic [3] and epigenetic changes [4]. Cancer cells develop self-sufficiency for proliferation and promoting signals and become insensitive to growth and proliferation inhibiting signals [5]. When normal cells face irreparable DNA damage, they stop dividing and undergo programmed cell death (apoptosis), while cancer cells become resistant to apoptosis [6]. Cancer cells also bypass the limits of replication potential through elevated telomerase enzymes and alternative telomere lengthening (ALT) and can replicate limitlessly even with errors in DNA [7, 8]. At each step of progression, cancer cells acquire additional changes to counteract new challenges like limited nutritional supply through processes of altered energy metabolism [9], angiogenesis [10], migration, and metastasis to more favorable locations [11]. Cancer is like an overall self-destructive program created by selecting robust abnormal cells in the body and can only be tamed to a large extent by therapeutic interventions.

Breast cancer is predominantly carcinoma of the cells of breast tissue arising as a result of uncontrolled cell division of epithelial cells of mammary ducts or acinar cells forming lobules of the breast. The breast is a dynamic organ in the sense that its growth is greatly influenced by reproductive hormones and can proliferate and differentiate for fulfilling the requirement of generating milk for a particular post-pregnancy window of reproductive life. The breast is a modified sweat gland composed primarily of fibrous and adipose tissues along with glandular epithelial tissue organized in the form of ducts and lobules. The basic unit of the mammary gland is acini that are lined by milk-secreting simple cuboidal cells surrounded by a layer of myoepithelial cells. Acini joined to form lobules that open into intralobular ducts, which will, in turn, empty into larger interlobular ducts finally into 15-20 lactiferous ducts to drain the milk through the nipple. Protective connective tissues surround lobules and ducts in the form of intra-lobular connective tissue and interlobular connective tissues giving support to glandular components of the breast.

Breast cancer is the second most common malignancy among women, accounting for 25% of the types of cancers [12]. In 2012 approximately 1.7 million new cases of breast cancer (11.9%) were reported and ranked as the fifth cause of death among cancer deaths (deaths, 6.4%). Affecting women in less developed regions (deaths, 14.3% of total), while the second cause of cancer death in more developed regions (deaths, 15.4%) [13]. One woman out of eight is at the risk of getting breast cancer [14]. According to the NCRP, 2012 report issued by ICMR, the estimated number of breast cancer cases in India was 144,937 (27%) in 2012 and ranked second after cervical cancer.

ORIGIN AND EVOLUTION OF BREAST CANCER

The initiation of breast cancer is the result of the accumulation of genetic and epigenetic changes and subsequent tumor progression is driven by clonal expansion and selection of mutated cells [15]. Accumulation of genetic mutations can lead to the change in internal signaling and normal cellular control in the breast. The continual replication of corrupted cells results in the formation of a colony of abnormal cells termed '*In situ* hyperplasia' which further leads to invasive carcinomas, and finally the metastatic form of the disease [16, 17]. Epithelial-mesenchymal interactions play a key role in the normal development of the mammary gland as well as for breast tumorigenesis [18]. *In vivo* and *in vitro* studies have demonstrated that the tumor microenvironment is composed of myoepithelial, endothelial cells, fibroblasts, myofibroblasts, leukocytes, and other cell types. The extracellular matrix (ECM) molecules regulate the tissue specificity of the normal breast as well as the growth, survival, polarity, and invasive behavior of breast cancer cells [19, 20]. Cytokines secreted by the resident or infiltrating cells of the tumor microenvironment could have a supporting or inhibiting role in the tumor growth and progression.

Sex steroid hormones play an important role in the development and physiological function of the mammary gland. Estrogens induce the proliferation of duct epithelial cells, blood vessel growth, and connective tissue while progesterone is responsible for tubulo-alveolar cell differentiation and development [21 - 24]. Androgens have been reported to suppress the proliferation of mammary epithelial cells [25 - 27]. Therefore, corresponding steroid receptors like estrogen receptor (ER) and androgen receptor (AR) can be strongly associated with the risk of initiation and progression of breast cancer. Recent reports suggest that AR is most predominantly (70-90%) expressed steroid receptors in a few subtypes of breast cancer [28 - 33]. The high level of estrogen among women is significantly associated with the risk of breast cancer [34 - 37]. Similarly, the level of androgen in postmenopausal women is associated with the risk of breast cancer [34, 38].

CHAPTER 3

DNA Damage Response: A Therapeutic Landscape For Breast Cancer Treatment

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Abstract: Breast cancer is responsible for cancer-related death among women globally. The known causes of breast cancer include genetic predisposition, dysregulated hormonal signaling due to psychological stress, and aging and lifestyle factors, such as smoking and alcohol consumption. Due to improved treatment strategies, the overall survival is significantly increased; however, it is still significantly associated with death worldwide. Breast cancer's initiation and progression are strongly influenced by genomic instability. Defect in DNA damage response (DDR) pathways, which enable cells to survive, help in the accumulation of mutation, clonal selection, and expansion of cancer cells. Germline mutation in breast cancer susceptibility genes, BRCA1 and BRCA2, TP53, and PTEN, increases the risk of early onset of disease. During the initial and clonal selection of cancer cells, a defect in one DNA repair pathway could potentially be compensated by another pathway. Therefore, cancer cells with defective DNA repair pathways could be easily killed by targeting the compensatory pathways by inducing synthetic lethality. Evidently, cancer cells with defective DDR or decreased DNA repair capacity show synthetic lethality in monotherapy when the backup DNA repair pathway is inhibited. For instance, tumors with defective homologous recombination (HR) can be targeted by inhibitors of double-strand break repair enzymes. Here, we briefly addressed the relevant factors associated with the development of breast cancer and the role of the DDR factor in the development of breast cancer. In addition, recent treatment strategies targeting genomic instability in breast cancer will be summarized as well as how the genomic instability and defective DDR can be targeted for the treatment of breast cancer.

Keywords: DNA damage, DNA damage response , DNA repair, PARP inhibitor, Synthetic lethality.

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INTRODUCTION

Breast cancer is a common cause of death associated with malignancy in women around the world.

In 2018, nearly 2.09 million cases of breast cancer in women were newly diagnosed and an estimated death occurred was 626,679 [1]. The graph of global incidence has been rising in breast cancer with an annual increase of 3.1%, beginning with 641,000 cases in 1980 and rising to more than 1.6 million in 2010 [2]; this trend still continues. Furthermore, the epidemiology of breast cancer requires more research as many countries register diagnoses and deaths but no relapses. One study reported that in 2017, the United States alone had approximately 160,000 cases of advanced-stage breast cancer [3].

The frequency of breast cancer incidence varies worldwide. The incidence rate is higher in high-income regions vis-à-vis low-income regions. The highest incidence rate is observed in high-income countries (HIC) such as New Zealand, North America, Australia, and northern and western Europe [4]. However, only 53% of all cases occur in less developed countries. In contrast to the increase in incidence, the mortality rate decreases in HICs, as cancer is usually diagnosed at an early stage, and with the availability of mammography, the prognosis is in the right direction. However, in low and middle-income countries the diagnosis is mainly as a late-stage often leading to poor survival. The mortality rate due to breast cancer is among the highest in many low and middle-income countries like the Caribbean islands (Bahamas), Pacific Islands (Fiji), Africa (Nigeria), sub-Saharan, and southern Asia (Pakistan) [5], despite their lower incidence. This is probably due to the late onset of the disease, and limited access to early detection and treatment.

Studies show that the median age of women when she is diagnosed is nearly 61 years with a peak range between 60 to 70 years in western countries; however, in Asian countries, the disease presentation is earlier, and the peak range is between 40 to 50 years [2]. In addition, the patients detected with breast cancer are almost 10 years younger in developing countries as compared to developed countries [6]. The biology of tumors also varies with ethnicity eg., African and African-American women are highly diagnosed with triple-negative breast cancer (TNBC) as compared to any other ethnic group [7]. They also represent increased cases of metastatic disease and the majority of poorly differentiated and undifferentiated grades among all subtypes, all of these conditions lead to lower survival. Moreover, 9% of breast cancers diagnosed in non-Hispanic black women are metastases which, in other ethnic groups, represent between 5% and 6% [8].

In men, breast cancer is a rare disease, accounting for nearly 1% of all breast cancers. According to the American cancer society, the risk of getting diagnosed breast cancer in men is 1 in 833. The risk is greater in black men as compared to non-Hispanic white men [9].

Risk Factors

Major risk factors include age, family history, alcohol consumption, reproductive factors (including nulliparity, early age at menarche, later menopause, and first childbirth after 30 years of age), physical inactivity, use of menopausal hormone therapy, and use of contraceptive pills [10, 11]. Obesity and being overweight are also linked with post-menopausal breast cancer. However, there is no such evidence that shows the effect of these factors on premenopausal breast cancer [12]. Furthermore, breastfeeding reduces the risk of having breast cancer [13]. A continuous event of lesions and accumulation of genetic modifications at the morphological level promotes a normal gland to cancer (Fig. **1**).

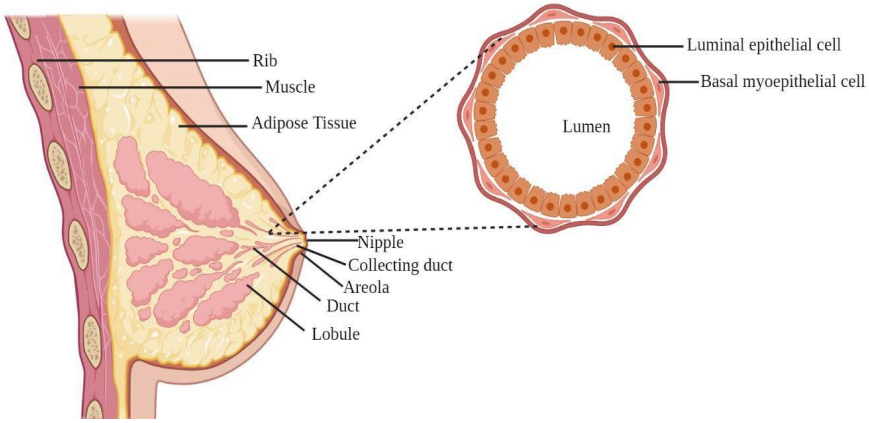

Fig. (1). Breast cancers originate in the epithelial cells of terminal ductal lobular units of collecting ducts.

SUBTYPES

The methods to classify breast cancer in different biological subtypes include molecular pathology, histopathology, genetic analysis, and gene expression profiling.

Histological Classification

Breast cancer can be broadly divided into *in-situ* carcinoma and invasive (infiltrating) carcinoma. Based on the pattern of growth and cytological characteristic features, breast *in situ* carcinoma is further categorized into lobular or ductal. Ductal carcinoma *in situ* (DCIS) is more common than lobular carcinoma *in situ* (LCIS) and comprises a heterogeneous group of tumors. DCIS

CHAPTER 4

Emerging Trends in Bioinformatics for Breast Cancer Molecular Research

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Abstract: Applications of bioinformatic methods and high-throughput sequencing techniques have increased exponentially over the last decade, transforming the way we understand complex malignancies such as breast cancer. In this chapter, an overview of recent advances in molecular research in breast cancer using emerging bioinformatics methods is presented. Learnings from scientific studies that have successfully integrated and interpreted massive amounts of data generated from various platforms (multi-omics data) using bioinformatics approaches are also outlined. Additionally, pan-cancer studies that help identify the differences and commonalities across multiple cancers are reviewed. We also discuss bioinformatics applications that transform the way we decipher the OncoGenomic landscape of breast cancer. Finally, this study also summarizes current publicly available bioinformatics tools and databases for breast cancer research.

Keywords: Bioinformatics, Genomics, High-throughput sequencing, Multi-omics, Next-generation sequencing, Pan-cancer analysis, Transcriptomics.

INTRODUCTION

In recent years, the advances in technology to generate and analyze large amounts of biological data have helped improve the clinical treatment and management of breast cancer [1]. However, the incidence rate remains a concern. For the past few years, breast cancer has been one of the most common cancers diagnosed among women globally, accounting for about 25% of cases worldwide [2]. In 2021, it was estimated that the USA alone would report around 280,000 new breast cancer cases in women [3 - 5]. According to ACS (American Cancer Society), the breast cancer mortality rate has decreased 40% from 1989 through 2017 compared to a 0.4% yearly rise until 1989 [3 - 5]. Although the 5-year survival rate currently stands at 90% for women with invasive breast cancer, 20-30% of patients are

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reported to display metastatic relapse over time [3 - 5]. The prognostic outcome of patients who initially present with breast cancer is dependent on many factors such as early detection, tumor stage at detection, breast cancer subtype, and biological characteristics of the tumor [3 - 5]. Moreover, breast cancer subtypes like triple- negative breast cancer (TNBC) have a high metastasis rate and shorter survival time. Recurrence of tumor after the initial clinical intervention, chemoresistance, and evolving genomic changes in cancer cells also pose severe challenges for the effective treatment of breast cancer [3 - 5]. The developments in sequencing technologies, specifically massively parallel sequencing and microarray technologies, have profoundly changed our understanding of breast cancer dynamics and led to significant breast cancer research advancements [6, 7]. Additionally, not only is data readily generated and analyzed, but it is now efficiently stored and shared between research groups. The rise in cloud technology presents the scientific community with an unprecedented opportunity to access large amounts of publicly available breast cancer data for an in-depth understanding of tumor cell biology [8]. Over the last decade, high-throughput sequencing data analysis using bioinformatics methods has led to promising molecular discoveries in the realm of breast cancer research (Table **1**).

Table 1. Key discoveries in the past decade.

Year	Key Discovery
2011	A large meta-analysis of more than 10,000 breast cancer female patients found that radiotherapy delivered after breast-conserving surgery significantly reduces the risk of recurrence and breast cancer mortality [9].
2012	An integrative breast cancer data analysis identified four main breast cancer classes based on genetic and epigenetic modifications. Multi-omics data found somatic mutations in three genes, TP53, PIK3CA, and GATA3 showed the highest incidence (>10%) in all breast cancers. Basal-like breast tumors were shown to share many molecular commonalities with serous ovarian cancer [10].
	A multi-omics approach combining the power of whole-exome DNA sequencing and whole-genome sequencing, Banerji et al., identified six genes (*CBFB*, *TP53*, *PIK3CA, AKT1*, *GATA3*, and *MAP3K1*) significantly mutated in breast cancer. The study, further, established the frequent involvement of AKT3 in recurrent genomic fusion events. These findings opened the door to investigating the role of AKT3 inhibitors for treating fusion-positive breast cancer subtypes like TNBC [7].
2015	Almost all cases of invasive lobular carcinoma (ILC), the second most prevalent histological subtype of invasive breast cancer, showed loss of CDH1 at DNA, mRNA, and protein levels. The Cancer Genome Atlas (TCGA) multi-omics study, also, identified significant mutations in PTEN, TBX3, and FOXA1, shedding light on the genetic signatures of ILC [11].
2016	National Cancer Institute (NCI) launched the largest-ever breast cancer study to investigate the inherited genetic variations associated with breast cancer in 20,000 black women. This study investigates the gene expression and genetic pathways involved in breast cancer [12].

(Table 1) cont.....

2017	Ogivri, the first biosimilar drug, was approved by the US Food and Drug Administration (FDA) for breast cancer treatment [13,14].
	A case series study using high-throughput sequencing data of metastatic breast cancer provided critical molecular insights into treatment-resistant metastatic breast cancer. Single-cell RNA sequencing was used to identify phenotypic characteristics of individual cancer subclones from the metastatic breast cancer samples [15]
2018	Landmark TAILORx study reported that 70 percent of women with early-stage breast cancer receive no benefit from chemotherapy and can be treated with endocrine therapy alone [16,17].
	TCGA published the Pan-Cancer Atlas, a comprehensive collection of research articles based on cross-cancer analyses using the TCGA cancer data to understand the oncogenic processes, pathways, and cell of origin patterns across multiple cancers [18].
2019	Hormone receptor-positive metastatic breast cancer showed complete regression after treatment with tumor-infiltrating lymphocytes reactive against specific mutant proteins [19].
2020	Researchers identified infiltration of tumor microenvironment with certain types of immune cells as one of the molecular changes that potentially can explain the exceptional response to treatment [20].

MULTI-OMICS DATA IN BREAST CANCER RESEARCH

Omics data facilitate an interface to study molecular dynamics of tumor cells from multiple levels - genomic, epigenomic, proteomic, transcriptomic, and metabolomic. Such high-dimensional data can provide a more accurate view of the driving mechanisms of a heterogeneous disease like breast cancer.

One of the major public repositories of multi-omics data very often used is The Cancer Genome Atlas (TCGA) which has over 20,000 samples (primary cancer and matched normal) that span 33 cancer types [21]. Breast cancer samples in TCGA have been classified, based on genetic features, into two invasive types; more common invasive ductal carcinoma (65-85% of all breast cancer) and invasive lobular carcinoma (ILC) (about 10% of all breast cancer) [10, 11, 22]. Other commonly used publicly available multi-omics data repositories include national center for biotechnology information's (NCBI's), gene expression omnibus (GEO) [23] and European bioinformatics institute's (EBI), BioStudies database (previous platform was ArrayExpress) [24]. All these sources allow the research community to access data generated experimentally from studies carried out worldwide. The data in these sources include microarray, metabolomic, proteomic, transcriptomic, genomic, and other biological data generated by high-throughput technologies [25, 26].

Molecular Subtyping of Breast Cancer Using Multi-omics Data

Breast cancer is a heterogeneous disease encompassing multiple molecular subtypes that present varied genomic profiles, clinical characteristics, histopathological features, and prognostic outcomes [27]. Gene expression studies

CHAPTER 5

Role of Nitric Oxide in Breast Cancer

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Abstract: Nitric oxide (NO) is a universal, water-soluble, free radical gas, which plays an important role in the physiological along with pathological processes. NO has been shown in the literature as a key player in carcinogenesis as well as tumor development. Still, there is a lot of debate and misunderstanding about its involvement in cancer. It is believed to have both tumoricidal as well as tumor-promoting effects, which are determined by its timing, location, and concentration. NO has been linked to angiogenesis, apoptosis, cell cycle, invasion, and metastasis. On the other hand, it is emerging as a possible anti-oncogenic agent. Strategies for manipulating *in vivo* production and exogenous delivery of this molecule for therapeutic gain are being investigated. For therapeutic advantage, strategies for controlling *in vivo* synthesis and exogenous distribution of this molecule are being investigated. Further research in experimental settings and clinical trials is required to enhance innovative NO-based cancer prevention and treatment strategies. The spectrum of NO actions in cancer and the mechanisms by which NO acts in breast cancer are addressed in this article.

Keywords: Nitric oxide, Nitric oxide synthase, Reactive nitrogen species, Tumoricidal.

INTRODUCTION

Cancer is the second leading cause of mortality in the world. Worldwide cancer accounts for about one in six deaths [1]. Globally, an estimated 1.5 million women are affected by breast cancer each year [2]. Breast carcinoma is the most frequently diagnosed malignancy and the second-most leading cause of cancer death among women.

Palmar discovered that epithelium cells synthesized nitric oxide from L-arginine in 1987 [3]. In mammalian cells, it is synthesized endogenously by the NOS (nitric oxide synthase) enzyme, is short-lived, and performs various physiological

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processes as signaling molecules. Nitric oxide gas produced in the body serves as a signaling molecule.

Unregulated and excessive nitric oxide production has been implicated as a cause of pathophysiological processes, including carcinogenesis. Nitric oxide synthase has been detected in many types of cancer like breast, uteri cervix, head and neck, laryngeal, and CNS (central nervous system) cancer [4 - 7]. Nitric oxide is thought to affect a variety of cancer-related processes [8]. Many research findings advocated that nitric oxide plays a dual role in carcinoma. At measurable levels in different types of clinical samples, nitric oxide supports tumour growth and proliferation. On the other hand, it has been demonstrated that nitric oxide has tumour effects. Many indirect and direct effect molecular mechanism pathways have been proposed for its antitumor characters [9, 10], but there is a lack of data on cancer patients. The tumoricidal characters of nitric oxide are being investigated for therapeutic purposes. Nitric oxide is used alone or in grouping with another cytotoxic agent [11].

Breast cancer is a complex malignancy with a heterogeneous expression of PR (progesterone receptor), ER (estrogen receptor), and HER2 (human epidermal growth factor) in patients, resulting in intratumoral and intertumoral heterogeneity, histology prognosis, and treatment responsiveness [12, 13]. The deviation in a transcriptional program, which could aid in providing a unique molecular profile for each tumor, is the main reason for the strong heterogeneity [12, 14]. Young women have been diagnosed with early breast cancer with an aggressive phenotype, necessitating the implementation of a breast cancer awareness and screening program [15]. An imbalance in the rate of production and removal of ROS (reactive oxygen species) or RNS (reactive nitrogen species) is known to cause oxidative stress. Reactive species can play a dual role, causing oxidative damage (as acting as molecular signals) and activating stress responses, all of which are beneficial to organisms [16]. Through pleiotropic effects on cell targets, reactive nitrogen species play an important role in cellular physiological regulation. A high level of RNS causes nitrosative stress, which has been linked to cell death and injury [17].

Nitric oxide acts as a signal molecule in different parts of the body as well as a cytotoxic or regulatory effector molecule in the innate immune system. Following enzyme activation of constitutively expressed eNOS (endothelial nitric oxide synthase) or nNOS (neuronal NO synthase), signal molecules of nitric oxide are synthesized on demand for brief periods (seconds to minutes). On the other hand, after cell activation, iNOS (inducible nitric oxide synthase) is expressed and produces nitric oxide for a long time (an hour to days). However, the controlled production of constant nitric oxide compared with the pulsative synthesis

distinguishes the pathophysiological and physiological action of nitric oxide.

All reactive nitrogen species (RNS) share a common, primary progenitor, and some RNS are made up of NO-dependent reactions. ONOO (peroxynitrite) and N_2O_3 (dinitrogen trioxide) are reactive nitrogen species that can cause oxidative and nitrosative chemical stress [18, 19]. A quick reaction between O^{-2} and NO produces $ONOOH^-$ (peroxynitrite), which is then converted into secondary RNS by another reaction. RNS reacts with nitric oxide (NO) and its intracellular environment to produce other reactive metabolites such as nitrite, S-nitroso-thiols, and peroxynitrite, all of which have genotoxic effects and cause DNA damage [20]. By targeting the sugar-phosphate backbone of DNA, peroxynitrite species can split the DNA into single-stranded [20].

Physiological and Biological Action of Nitric Oxide

Nitric oxide plays a crucial role in various biological processes like macrophage-mediated immunity, vasodilatation, and neurotransmission. Nitric oxide is a great reactive diatomic and diffusible free radical and is present at room temperature in gaseous form and has pleiotropic functions. Moreover, it can act as a messenger of molecules and participate in promoting and inhibiting carcinoma [12, 20]. Nitric oxide is a short-lived endogenously produced gas that acts as a signaling molecule in the body. It is synthesized by the nitric oxide synthase (NOS) enzyme, which is endogenously produced by mammalian cells at an appropriate time and magnitude. Overexpression of NO synthesis has been associated with many pathophysiological conditions including carcinoma. NOS expression is identified in several cancers like breast, uteri cervix, CNS (central nervous system), head and neck, and larynx cancer [4, 19]. NO has been shown to modulate various carcinoma-associated events [8]. NOS enzyme is ubiquitously expressed in cancer and helps in the NO synthesis in the presence of O_2 from L-arginine [12]. NOS required FMN, FAD, NADPH, and BH4 [(6R-) 5, 6, 7, 8-tetra-hydrobiopterin] as cofactors. NOS1, NOS2, and NOS3 are three distinct genes encoding isoforms of NOS in mammalian cells, with 51-57 percent homology in regulation, localization, inhibitor sensitivity, and catalytic properties. NOS1 is classified as an isoform first purified and cloned from neuronal tissue (nNOS), while the isoform first found in endothelial cells (eNOS or NOS_3) is known as constitutive because it is expressed continuously in endothelial cells and neurons, respectively. Multiple factors, including interferon (IFN), interleukin (IL-1), tumor necrosis factor (TNF), oxidative stress, and bacterial endotoxin, can inhibit or trigger eNOS and nNOS expression through protein kinase-mediated phosphorylation whereas iNOS expression can be regulated transcriptionally by multiple factors, including interferon (IFN), interleukin (IL-1), tumor necrosis factor (TNF-α) and bacterial endotoxin such as lipopolysaccharide (LPS) [12].

CHAPTER 6

Autoantibodies as Clinical Biomarkers in Breast Cancer

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Abstract: Breast cancer (BC) is one of the most diagnosed and worldwide malignancies in females with an estimated 1,300,000 new cases and 465,000 deaths annually. Therefore, early diagnosis and effective treatments of BC are urgently needed in the struggle against this disease. Molecular markers research has gained huge momentum in BC management. Very few molecular markers are in clinical use for BC management. However, owing to BC heterogeneity, more molecular markers are required for better diagnosis and treatment. Humoral immune response defines the generation of autoantibodies (AAbs) in blood against tumor-associated antigens (TAAs). Such AAbs have been showing great promises for biomarker development for cancer detection. Therefore, these candidate AAbs might be useful for developing blood-based detection assays along with other existing diagnostic tools for BC patients. Besides that, AAbs can also assist in the identification of novel TAAs that can further enhance the utility of immuno-proteomics for biomarkers development and targeted therapy. In this scenario, proteomics tools are being extensively utilized to identify novel TAAs.

Keywords: Autoantibodies , Autoantigens , Biomarkers , Early detection of cancer , Tumor-associated antigens .

INTRODUCTION

Biomarkers

Biomarkers are defined as biomolecules that can be found in biofluids or tissues and can predict the occurrence of any condition normal or abnormal like disease onset, disease progression, and treatment response. Any type of biomolecules can serve as biomarkers if they can differentiate between normal *vs.* abnormal conditions with certain specificity and sensitivity [1]. Several biomolecules like cells, Proteins, nucleic acids like RNAs and DNAs, metabolites, and antibodies

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have been defined in the literature so far. Biomolecules can be found in any biological material like blood, stool, urine, nipple discharge, or tissues. Biomarkers can be accurately identified in diseased conditions and can work as-clinical biomarkers as they can be utilized for diagnosis and prognosis of the disease This ability of biomarkers has been appreciated well in cancer research as several biomarkers have been known to clinically diagnose cancer at early stages. Biomarkers are also useful to monitor therapy response and recurrence of cancer.

Breast Cancer Biomarkers

Breast cancer is a heterogeneous disease defined by various subtypes. Heterogeneity in breast cancer led to the discovery of various biomarkers which are guiding breast cancer treatment. The most known are estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). Based on these three biomarkers, breast cancer treatment has been revolutionized. Other than these, the concentration of glycoproteins like CA15.3 and CA27.29 are also helpful in monitoring disease progression [2]. Since the discovery of these protein markers, many other markers have been explored for their utility in the diagnosis or prognosis of breast cancer, however none of them have reached the clinic to date [3]. However, consistent efforts have led to the discovery of other potentially useful markers. In this consistent effort of biomarker findings, autoantibodies (AAbs) emerged as an important tool for the early diagnosis of breast cancer [4].

AUTOANTIBODIES

Autoantibodies (AAbs) are the antibodies against self-antigens in the body. AAbs generation is a natural process in the human body. Healthy human beings contain AAbs against several self-antigens which may take part in the natural defense system. Self-antigens are proteins that trigger the generation of antibodies against antigens. However, any change in self-antigens like antigen alteration by mutation, post-translational modification, overexpression, or release of intracellular antigen upon cell lysis, can further trigger the generation of AAbs against self-antigens [5]. During the early events of tumorigenesis, many antigens change their natural behavior as they either show overexpression or get mutated which changes their confirmation and exposes new sequences for antibody generation [6]. Such changes can trigger the generation of AAbs during the early events of tumorigenesis and thus can mark the beginning of cancer in the human body.

GOALS OF CANCER BIOMARKERS

The idea of biomarker discovery circumvents such markers which have the

following characteristics:

1. Help in the development of non-invasive that can diagnose cancer risk in its earliest stages.
2. Can categorize cancer in a way that can guide appropriate therapy and disease progression.
3. Which are stable in easily accessible samples like blood.
4. Which can easily get identified in samples with the available tool.
5. Which are less prone to proteolysis during the sample collection, storage, and processing.
6. This can help in the development of a cost-effective test.
7. Which showed better sensitivity and specificity for the diagnosis of the disease.

Thus, a plethora of search work has been done and still going on to find out such biomarkers in human blood which can fit into all these criteria.

AUTOANTIBODIES AS POTENTIAL CLINICAL MARKERS FOR CANCER

Blood plasma is the most preferable choice of sample for the identification of Tumor-associated antigens (TAAs). However, there is poor detection of antigens themselves in blood as they are present in very low concentrations in blood so the current methodology is not able to identify them. Furthermore, antigens have a short life in blood and are highly prone to proteolysis. Due to such discrepancy, AAbs seem to be the better option for biomarker discoveries than their antigens themselves.

Cancer triggers an immunologic response in the form of AAbs against TAAs. AAbs pose immense potential for early diagnosis of breast cancer. As AAbs are less prone to degradation and more stable in blood. They also show persistent responses over time as their self-life is more than TAAs. Furthermore, AAbs can be easily detected in blood by the recently available, cost-effective techniques. Besides this, the most important point of consideration for AAbs is that they produce in very early events of tumorigenesis and can be detected several years before the clinical development of cancer [7]. Therefore, AAbs can become the obvious choice for the detection of early-stage breast cancer.

To show the clinical utility as a diagnostic biomarker, AAbs should be able to distinguish breast cancer patients with high accuracy, specificity, and sensitivity. To become a good early screening marker, certain cutoff values for the AAbs should be selected to determine diagnostic accuracy in the form of high sensitivity and specificity. Diagnostic accuracy can be plotted in a receiver operating

CHAPTER 7

Epigenetics of Breast Cancer

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Abstract: Breast cancer is a very heterogeneous disease at clinical, histological, and molecular levels. It is the leading cause of cancer-related deaths among women. Breast cancer is manageable if diagnosed early at a localized stage, but late diagnosis of metastatic disease has a very low patient survival rate. Further, limited treatment options, insufficient prognostic and diagnostic markers, misdiagnosis and drug resistance pose a greater problem for patient survival and clinical outcome. Consequently, there is a great need to explore newer and more effective diagnostic, prognostic and therapeutic options for managing breast cancer. It is now a well-known fact that along with genetic changes, epigenetic modifications play an important role in the origin and pathogenesis of breast cancer. Universal involvement of epigenetic modifications in breast cancer development makes them useful for diagnosis, prognosis, and follow-up purposes. Further, the reversibility of epigenetic changes makes them attractive targets for breast cancer therapy. Therefore, in this chapter, we will discuss current knowledge on epigenetic involvement in the development of breast cancer and epi drugs as treatment options for breast cancer management.

Keywords: DNA methylation, Epigenetics, Histone deacetylases , Methyltransferases , microRNAs .

INTRODUCTION

Cancer evolution is a multistep, complex process driven by various genetic and epigenetic abnormalities in the cell [1]. Genetic abnormalities involve gene mutations, chromosomal alterations which can be structural or numerical and which are rare in normal cells but are prominent in the tumor cells [2]. Genetic abnormalities are heritable changes in DNA sequences that can alter the gene

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expression and genomic stability in a cancer cell, whereas epigenetic changes are heritable, which alters the expression of genes without altering the DNA sequence [3].

Epigenetic changes cause silencing of tumor suppressor genes (TSGs) and activation of oncogenes in a cancer cell [4]. Epigenetic changes involve DNA methylation which occurs at the 5th position of cytosine at CpG dinucleotide sequences and modification of histone proteins by covalent changes like phosphorylation, acetylation, and methylation, which alter cellular gene expression without changing DNA sequences [5]. In the past two decades, microRNAs, a group of noncoding RNA, have also been accepted as an epigenetic modulator as these regulate post-transcriptional modification [6, 7]. There are more accepted and recently described modifications, including nucleosome positioning, chromatin remodeling, and chromosomal looping [8 - 10]. All epigenetic modifications are strongly linked, and can easily induce one another [8]. Local CpG hypermethylation at promoter regions silences TSGs expression, whereas global hypomethylation increases genetic instability. Histone modifications affect the packing of genetic material which consequently alter gene expression by changing the accessibility of transcription factors at promoter or enhancer sites [11, 12]. Among all types of cancers, breast cancer is the most common type of cancer among women and the second most common cancer after lung cancer. Breast cancer affects millions of women worldwide. It is a leading cause of cancer- related deaths among women and causes a significant economic burden [13]. In recent years, various diagnostic and treatment options have been explored to manage localized and metastatic breast cancer. Combined epigenetic therapies show enormous potential in the management of breast cancer. Tremendous efforts have been put forward by researchers in the past decades to resolve the intricate relationship of epigenetics involvement in the development and progression of breast cancer. But still, there is a massive gap in the understanding of complex interactions between epigenetics modifications and breast cancer. Therefore, a better understanding of breast cancer epigenetics is needed to explore its immense potential to manage breast cancer. In this chapter, we will discuss epigenetic modifications, their involvement in breast cancer pathogenesis, and exploiting epigenetic modulation as treatment options.

EPIGENETIC PROGRAMMING AND CELLULAR PHYSIOLOGY

Epigenetic changes are important for the development, maintenance, survival, and specific functions of a cell. Some important developmental events like patterning by Hox genes, X-inactivation, neuronal development, and genomic imprinting are regulated by epigenetics [14]. Each and every cell has its own epigenetic blueprint which is then inherited by its progeny. DNA methyltransferases (DNMT's) and

histone modifiers (Table **1**) maintain these blueprints during cell division and restore the function of specific cell types or tissue [3]. Epigenetic imprinting also functions as an epigenetic memory for a cell [15]. Various molecules are involved in maintaining the epigenetic makeup of a cell. DNA Methyltransferases (DNMT1, DNMT3a, and DNMT3b) are enzymes that methylate DNA at the CpG dinucleotide sequence. DNMT1 is the maintenance enzyme that regulates the methylation blueprint of a cell after replication whereas DNMT3a and 3b are de-novo methyltransferases that generate new methylation patterns and actively participate in developmental processes [16].

Table 1. Epigenetic regulators that maintain epigenetic blueprint of a cell.

Epigenetic Modification	Category of Enzymes	Enzymes	Function
DNA modification (DNA methylation)	DNA methyltransferases	DNMT1, DNMT3a/3b/3l	Gene silencing, chromatin organization, stable heritable modification, X-chromosome inactivation, imprinting, silencing of repetitive elements
	DNA demethylases	TET, AID, MBD2/4	
Histone modification (Methylation Demethylation Acetylation Deacetylation)	Histone methyltransferases (HMTs)	PRMTs, EZH2, SUV39h1, SYMD3	Heritable labile modification, activation or repression of gene transcription, chromatin structure and nucleosome positioning
	Histone demethylases (HDMs)	LSD, JMJD, JDHM	
	Histone acetyl transferases (HATs)	Tip60, MOZ, MORF, CBP, p300	
	Histone deacetylases (HDACs)	HDAC1-11, SIRTs	

Methyl binding domain proteins (MBD's- MBD1, MBD 2, MBD 3, MBD 4, and MeCP2) read methylation marks and help to make repressor complexes [17 - 19]. Histone proteins maintain chromatin structure and its dynamics. Histone modifiers act on amino acids at the histone tail and change their affinity toward chromatin. Histone modifying enzymes are histone acetylases, histone deacetylases (HDACs), histone methyltransferases (HMTs), phosphorylases, and sumoylation enzymes. Polycomb proteins that were identified in Drosophila make repressor complex PRC1 and PRC2 which modifies histone at specific positions and direct DNA methylation. EZH2 is an important member of PRC2 and functions as histone methyltransferases [20 - 22].

Epigenetic Modifications are Strongly Interlinked

Although it has been observed in the in-vitro system, DNA methylation can alone

CHAPTER 8

Nanoparticles Targeting and Uptake: Current Advances in Breast Cancer Research

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Abstract: With the rapid advancement, nanoparticles (NPs) based drug delivery systems have been recognized as expedient over traditional therapeutics for breast cancer, fostering targeted drug release, long circulation time, reduced toxicity, and greater bioavailability. Under normal circumstances when this exogenous structure of nano-scale dimension approaches nearby cells, it evokes early tripping leading to membrane wrapping and NPs cellular uptake. Tailoring NPs structure for safe and intended entry into cells is at the core of nano-therapeutics for attaining high-yield prognostic and therapeutic efficacy. Interestingly NPs uptake is crucial as it unravels pathway selection and is decisive for the intracellular fate of nano-medicine. Over the past, it remained a major challenge to target specifically to improve their delivery. A significant effort has been devoted to understanding the endocytosis of nano-medicine for efficient intracellular delivery of NPs. Here we present an overview of the different endocytic pathways used by cells. Novel strategies in NPs design to exploit the uptake mechanisms to decipher intended uptake and target breast cancer. Current advances and strategies are deployed to breach these barriers and attain the ultimate vision of nano-carriers in diagnostics and therapeutics.

Keywords: Endocytosis, Nano-carrier, Nanoparticles, Targeted Therapeutics.

INTRODUCTION

Breast cancer is the primary reason for the growing fatality rate among all cancers in females around the world with close to 60% of deaths taking place in developing Countries [1]. According to recent reports, breast cancer as of today is the most commonly visualized in both developed and developing countries [2]. Incident rateswere recorded in different parts of the world with varying rates. Per 100,000 women19.3 cases were recorded in Eastern Africa and 89.7 cases in Western Europe with an overall average of greater than 80 in developed regions

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of the world and 40 in developing regions. This will increase the world's breast cancer burden in the future. Collective efforts are underway to lessen the global incidence of disease and increase the survival rate in breast cancer incidences with cost-effective interventions. Varying contemporary medicines are recommended to cure breast cancer, but the main impediment associated with this is its heterogeneous nature encompassing multiple subgroups with different cellular composition, molecular signature, prognoses, dissemination patterns, and responses to therapies. The nanoparticles (NPs) carrier system represents a promising therapeutic vehicle for the transportation of anticancer drugs in diseases like breast cancer. However, the main challenge encountered in the delivery system is the targeted delivery to the specific cancer site within the complex tumor microenvironment. These nanocarrier systems due to their unique size to surface ratio show enhanced drug-carrying capacity, controlled drug release profile, and overcome various biological barriers to confer target drug delivery. These tailored NPs include therapeutic compounds loaded in NPs, with increased release kinetics, and are expected to be released intracellular to achieve efficacy. Because of their unique size, though NPs act as a good delivery carrier, but the poorly organized vascularization system, and increased pressure within the tumor microenvironment limits the easy entry of particles inside the system. Hence targeting specific cells and uptake efficiency of the delivery vehicle into the tumor cells is imperative for predicting the therapeutic performance of nano-medicine. To address this major quest various targeted delivery of nano-therapeutics has been designed to confer enhanced circulation time, cellular uptake, improve therapeutic efficacy, and decrease systemic toxicity. Here the current developments in targeted nano-carrier systems specifically for breast cancer are envisioned with special emphasis on different endocytosis mechanisms.

INTERNALIZATION PATHWAYS

NPs-based drug delivery strategies have arisen as a desirable vehicle for drug transport throughout the body by overcoming the problems associated with conventional drug formulations. However, prior to targeted delivery of any therapeutic, it is a prerequisite to overcoming several transport barriers for efficient accumulation of nano-therapeutics at the diseased location. To achieve a proper therapeutic outcome, the transport of NPs across the plasma membrane has been imperative. Substantial research efforts have aimed to understand the detailed mechanism of NPs internalization for competent cargo transport. Plasma membraneforms a barrier from the surrounding environment and provides a protective milieu for various physiological processes. Apart from this, it plays a role in cellular adhesion, endocytosis, and communication. Endocytosis largely encompasses intracellular membrane-enclosed vesicles formed mostly by the cell

membrane invagination made up of internalized cargo along with extracellular fluids. When NPs arrive at the surface of cells, they maintain contact with various constituents of the plasma membrane and through endocytosis are internalized by the cells. It is frequently observed that the low molecular weight and hydrophobic molecules easily permeate across the membrane; however, micro-and nano-particles employ active internalization procedures [3]. In the case of NPs receptor-mediated endocytosis is the main uptake pathway, which is governed by the binding of cell surface receptors with molecules linked to the surface of NPs. Endocytosis is a very dynamic and regulated process with different variants being dependent on the cell type and cargo transported. Several uptake processes are present for the cellular internalization of NPs including phagocytosis, clathrin, caveolae-dependent, clathrin- and caveolae independent endocytosis and macropinocytosis but broadly internalization by means of endocytosis is of two types of namely phagocytosis and pinocytosis. Prior to the strategies in designing targeted nano-medicine capable of efficiently targeting breast cancer a detailed understanding of the different endocytic pathways is needed. Hence, comprehensive overviews of the different endocytic pathways are described below.

PHAGOCYTOSIS

Endocytosis mediated through phagocytosis is a characteristic of professional phagocytic cells like macrophages, dendritic cells, monocytes, and neutrophils, employed in host defense mechanisms associated with both innate and adaptive immunity and clearance of cell debris. Apart from these, a few other cells also show phagocytosis (fibroblasts, basophils, eosinophils, mast cells, natural killer cells, epithelial and endothelial cells) but only to a very low extent [4, 5]. Phagosomes formed for the engulfment of any entity can span up to >250 nm [6]. Phagocytosis can be stimulated either through the interaction of ligands already present on the surface of a foreign entity with cell surface receptors or soluble factors that bind and help recognize that entity to cells (opsonization). Mostly in the case of NPs phagocytosis is triggered through opsonization of soluble factors which includes complement proteins, immunoglobulins (antibodies), or other blood proteins (acetylcholine, fibronectin, laminin, c-reactive protein, and type-I collagen) [7]. Mostly the receptors involved to elicit phagocytosis are Fc receptor family (FcγRI, FcγRIIA), mannose/fructose receptor, complement receptors (CR1, CR3, and CR4), scavenger receptor, and α5β1 integrin receptor [8].

Processes involving NPs internalization *via* phagocytosis have been extensively explored to design particles for regulating phagocytosis and intracellular targeting. Recently it was found that particle geometry has been a major impetus for internalization *via* phagocytosis [9]. Particles assisting surface receptor-

CHAPTER 9

Dietary Polyphenols and its Molecular Mechanism in the Management of Breast Cancer

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Abstract: Despite clinical and pharmacological advancement in medical science breast cancer has become a global concern due to the high mortality rate. Breast cancer is mainly associated with altered redox status, cell cycle, chronic inflammation, and increased proliferative rate. Breast cancer has various molecular subtypes and adequate knowledge of these altered cell cycle regulatory cascades and molecular subtypes of breast cancer is a must for proper prognosis and its successful treatment. The discovery of drugs with anticancer properties, particularly against the specific subtype of breast cancer has become a challenging task for cancer researchers. Dietary polyphenolic compounds as cancer chemopreventive agents have drawn much attention among researchers because polyphenolic compounds are natural in origin with lesser side effects and have a wide range of action against various subtypes of breast cancer. Dietary compounds with antioxidant properties have been reported to act on an array of genes and proteins associated with breast cancer pathogenesis and thus regulate the signaling cascade related to autophagy, chronic inflammation, apoptosis, and cell cycle regulation. All in all, these natural compounds regulate growth and progression of a tumour with less or no side effects. Thus, the current article focuses primarily here on various aspects of breast cancer and food polyphenolic compounds as wellas their molecular mechanism for managing breast cancer.

Keywords: Epigallocatechin gallate, Polyphenols compounds, Quercetin, Resveratrol, ROS.

INTRODUCTION

In the current scenario, cancer has become one of the most common life-threatening evils across the globe. Breast cancer is most common among women and each year out of 1.4 million breast cancer incidents 450000 leads to death. It

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is assumed that one in eight women will have breast cancer during their lifespan [1]. There are four universal strategies to regulate different stages of carcinogenesis [1 - 3]. The first one is the cancer chemopreventive approach which effectively modulates the carcinogenic and mutagenic effect and-consequently, inhibits initiation and promotion steps in tumor formation. The second and most effective approach is to involve such a strategy that prevents early stages of carcinogenesis *via* modulation of signal transduction, blocking angiogenesis, antioxidant mechanisms, and altering immunity, which finally results in the blockage of cancer progression and chemoprevention. The third strategy to tackle cancer treatment is to control the metastatic potential in the tumor by targeting the proteins and genes such as regulating the epithelial-mesenchymal transition in the tumor [1, 2]. The fourth one is the surgical approach for breast cancer, which is very invasive and exhibits the probability of cancer recovery.

Cancer researchers are in search of promising and targeted drugs having lesser side effects, for breast cancer management [4]. Dietary compounds which are secondary metabolites from different sources such as plants [5], microbial [6], and marine species [7] have proved their efficacy against various types of cancer including breast cancer and other types of disease and disorder. Among various dietary compounds, the polyphenols have emerged as one of the promising compounds having great potential for the management and prevention of various types of disease and disorders such as cancer [8 - 13], diabetes [14, 15], inflammation [16 - 19], obesity-associated diseases [20], neurodegenerative disorders [21 - 23], bacterial [24 - 27] and viral infections [28, 29] or cardiovascular diseases [30]. In addition, these polyphenolic compounds have potent antioxidant activity and can target an array of genes and proteins and thus signaling cascade altered during carcinogens and various types of neurological and inflammatory disorders [31 - 35].

Polyphenolic compounds are found in high concentrations in fruits, vegetables, tea, spices, and their molecular structures are characterized by the presence of one or more phenolic rings substituted with at least one hydroxyl group. Phenolic acids, flavonoids, and lignan stilbenes are the main group of phenolic compounds. Polyphenols have potential anticancer activity, including breast cancer, they are multifaceted compounds and have been demonstrated to target an array of genes and proteins associated with apoptosis, metabolic pathway, angiogenesis, epithelial-mesenchymal transition (EMT), and cell cycle regulatory as well as inflammatory cascade [36]. The multi-targeted activity of these dietary polyphenols against cancer, including breast cancer is mainly due to their antioxidant activity. This antioxidant activity imparts and alteration of an array of proteins, enzymes, and membrane receptors, resulting in regulating gene

expression, apoptosis induction, vasodilatation, and modulation of the cell cycle in cancer [37 - 42]. In addition, these dietary polyphenols have cancer chemopreventive effects against tumor initiation through numerous mechanisms, such as the inhibition of carcinogenic molecule formation, blockade of oncogenic transforming enzyme activity [43], regulation of phase I and II enzymes, such as cytochrome P450s(CYP) [44] and S-transferase (GST) [45], as well as preventing DNA damage in the cells [46, 47]. For all these reasons, new treatments based on polyphenolic compounds are being studied as an alternative and/or adjuvant therapies in these pathologies using different breast cancer models [21]. The potential benefits of their dietary intake on human health and, more specifically, on cancer risk (including breast cancer) have been also reviewed [48, 49]. Specifically, for breast cancer, interesting results have been obtained with a mixture of tea extract and quercetin [50], with Pinus radiata [51], Indian lotus [52], *Hypogymniaphysodes* lichen [53], *Morindacitrifolia* [54], or with olive leaf extracts [55 - 58], among others. In this book chapter, we will discuss the types of breast cancer, including biomarkers associated with it and therapeutics associated with it.

Breast Cancer: An Overview

Breast cancer is a more dreadful and heterogeneous group of diseases and accounts for the second most diagnosed cancer after lung cancer. As compared to the global rate of incidents of death associated with cancer, breast cancer ranks the fifth position among all types of cancer. According to a Globcan report, 1.7million women were diagnosed with breast cancer in 2012, and in previous years, the incidence of breast cancer has increased by 20%, while the rate of mortality has increased by 14%. Moreover, breast cancer is the most common cause of death and frequently diagnosed cancer in women among 140 out of 184 countries worldwide. Although breast cancer is the frequently diagnosed cancer in developed countries, Globocan's cancer data also add a high mortality rate in developing or less developed countries. The incidence of breast cancer is to some extent because of changes in lifestyles, as well as the lack of clinical advances to breast cancer patients living in these regions. The current data on breast cancer evidenced that the rapid social and economic changes, the altered lifestyles of industrialized countries lead to an increased burden of cancers associated with reproductive, dietary, and hormonal risk factors may be the stipulated reason for increasing trends of breast cancer incidence among developing countries. The comparative analysis of data among developed and developing countries showed that the incidence rates remain highest in more developed regions, but mortality is relatively much higher in less developed countries, which is due to a lack of early detection and access to treatment facilities.

CHAPTER 10

Radiotherapy in Carcinoma Breast

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Abstract: Radiotherapy therapy is one of the effective and curative methods for the treatment of cancer. One of the reasons for the growing popularity and increased outcome of radiotherapy is attributed to the tremendously enhanced capacity of detection and imaging quality with the reduced radiation dose. Breast cancer is the leading cause with the highest percentage incidence in women worldwide and is the leading cause of cancer death, especially in the developing world. Over 50% of breast cancer patients have been prescribed radiotherapy during their cancer disease management. The present chapter discusses a comprehensive approach to the role of radiotherapy in breast cancer, including the theory, different phases, and types, clinical aspects as well as the challenges involved in its optimal outcome. Chemotherapy, hormone therapy, *etc*., are the primary treatment modalities for breast cancer, outside of surgery. In this chapter, external beam radiation treatment is mainly discussed.

Keywords: 3-Dimensional conformal radiotherapy, External Beam Radiotherapy, Intensity-modulated radiation therapy, Medical LINAC, Virtual Simulation.

INTRODUCTION

Radiotherapy therapy is one of the effective methods for the treatment of cancer. In this process, ionizing radiation is delivered with the primary intention to kill the tumor cells sparing the normal cells as much as possible taking into account tumoricidal and tissue tolerance dose [1, 2]. With continuous technological improvement in cancer treatment, high-energy x-ray and gamma photon beams of the order of MeV or MV are being used. Apart from its use for the treatment of cancer cells, radiotherapy is also useful for a few non-malignant benign conditions. Sometimes it is used in combination with surgery, chemotherapy, or hormone therapy [3, 4]. Broadly radiation therapy can be divided into two categories *viz* External Beam Radiotherapy (EBRT) and Brachytherapy (BT). The

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most common radiation beam used in EBRT is photons, but it can be of electrons, heavy ions, or some heavy particulate radiation. Radiotherapy is given either-with curative intention or with the primary aim to relieve the pain and symptoms as well as to enhance the quality of life; commonly known as palliation [5, 6].

RADIOTHERAPY: DEFINITION AND PRINCIPLE

Radiotherapy is a branch of medicine that utilizes ionizing radiation for the treatment of tumor and occasionally benign diseases, as well. Due to the hazardous nature of ionising radiation, it is delivered in a very controlled and precise manner to achieve its principle that demands maximum dose delivery to the target volume with as low as possible dose to the surrounding normal structure/normal cells. It is basically a differential response of tumor cells and normal cells towards ionizing radiation that allows the use of radiation in the treatment of cancer diseases. This fact can be understood with the help of the diagram (for the depiction only) shown in Fig. (**1**).

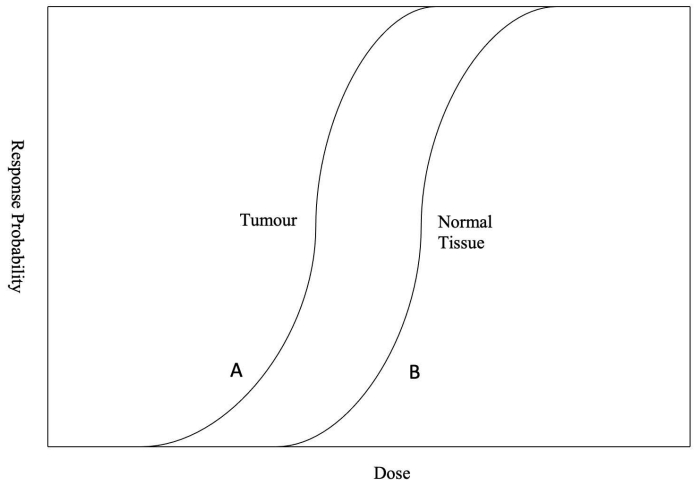

Fig. (1). Relationship between the response of both tumor, normal tissue, and radiation dose (Not to scale).

The dose range/interval between two points A and B on the dose axis are called a window where A is the dose point beyond which tumor irradiation/tumor control starts and B is the point where normal tissue complications start. The success of radiotherapy depends on this window gap also, majorly, with a wider window higher dose can be delivered to the tumor volume without having severe side effects of radiation.

As soon as radiation comes into the vicinity of human tissues, it starts to interact with the medium. In fact, radiation beams damage the deoxyribonucleic acid (DNA) of malignant cells. The double-strand break is considered to be the

endpoint, making cells undergo apoptotic mode. Once damage to the DNA of the tumor cell is achieved, then its replication is halted [7]. Sometimes the DNA of a cell is not completely or precisely damaged, resulting in a mutation.

The irradiated volume in a patient usually contains both targeted healthy and tumor tissues. DNA damage occurs in healthy cells as well as tumor cells but due to faster reproduction in normal cells and reduced repair capability of tumor cells, they are more sensitive to ionizing radiation [8, 9]. Normal cells have better capabilities to handle the damage caused by radiation exposure compared to malignant cells for the same level of damage and this improves drastically if given the adequate time provided the dose is within the tolerance limit. Thus, the success of quantifiable radiation therapy in terms of therapeutic gain (equation 1) is the direct result of the exposed dose [10, 11].

$$Therapeutic\ gain = \frac{Tumor\ Control\ Probability}{Normal\ Tissue\ Complication\ Probability} \quad \textbf{(1)}$$

Tumor control probability (TCP) is defined as the probability of eradication of tumor cells from the cell population, whereas normal tissue complication probability (NTCP) gives the probability of fatal damage to normal cells from the same cell population. As depicted in Fig. (**1**), these two probability curves lie very close suggesting a very small dose difference for favorable response both from the tumor and normal cells at the same time. This means that radiation treatment requires a very high degree of precision. Therefore, the optimal radiation dose should be administered in a way that maximizes the TCP while minimizing NTCP [12, 13].

RADIATION SOURCES IN EXTERNAL BEAM RADIOTHERAPY

As described in the section, two approaches are used, in general, in combination or alone *via* external beam radiotherapy (EBRT) and internal beam radiotherapy or brachytherapy (BT). Here in this chapter, we would be restricting to EBRT, in which radiation source is maintained at some distance from the tumor. The external beam radiotherapy units (in convention) can be (i) Radioisotope based *e.g*. Co-60 and (ii) artificial radiation units such as Medical LINACs (Linear Accelerators).

Telegamma (CO-60) Unit

The history of radiation application in cancer therapy is as old as the discovery of x-rays. Many of the artificial radiation sources have been developed from time to time for cancer therapy and the same has been obsolete due to some reasons. Like

CHAPTER 11

An Overview of Breast Cancer Therapy

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Abstract: Breast cancer is the most common type of cancer among females worldwide. It is a heterogeneous disease where the treatment strategies depend on several factors, such as tumor stage, menopausal status, breast cancer oncogenes (BRCA1 or BRCA2), and hormone receptor (ER, PR, and HER2) status. Treatment of breast cancer may be neoadjuvant therapy when given before surgery or adjuvant therapy when given after surgery. Adjuvant therapy is also known as systemic therapy, where the cancer cells are treated with chemotherapy, radiotherapy, hormonal therapy, and immunotherapy. In this article, we present current therapeutic strategies and discuss the types of treatments that constitute the standard of care for breast cancer.

Keywords: Hormonal therapy, Immunotherapy, Neoadjuvant therapy, Radiotherapy, Systematic therapy.

INTRODUCTION

Breast cancer is one of the common types of cancer that affects women worldwide and is a major cause of death among women. It accounted for approximately 6.6% of the total number of cancer deaths in 2018 [1]. However, the incidence of new cases of breast cancer has significantly increased in the last 25 years, and the mortality rates have also increased, especially in developing countries and low-income countries, while high-income countries recorded low mortality rates [2]. On the other hand, breast cancer in men accounts for 0.8%–1% of all breast cancers [3].

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Breast cancer is classified into four major subtypes according to hormone receptors (HR) status including luminal A (HR+/HER2−), luminal B (HR+/HER2+), HER2+, and triple-negative (HR−/HER2−).Two subgroups (Luminal A and B) are hormone receptors positive (estrogen receptor (ER) and progesterone receptor (PR) breast cancer. Luminal A subgroup (HR+/HER2−) is usually less aggressive than other subtypes. The Luminal B subgroup (HR+/HER2+) has a high expression of Ki67 (a proliferation marker) so Luminal B has a relatively poor prognosis. HER2+ breast cancer has overexpression or amplification of the HER2/ERBB2 oncogene and may be treated with anti-HER2 therapies. While triple-negative breast cancer (TNBC) is usually more aggressive than hormone receptor-positive breast cancer subtypes [4]. The current breast cancer therapeutic strategies include surgery, radiation therapy, chemotherapy, and hormone therapy, or the combination of all the standard therapeutic strategies [5], as well as immunotherapy [6]. The choice of therapy depends on tumor size, tumor subtypes, tumor stage, age, menopausal status, genetic factors, hormone receptor status, and HER2 status. Therapy planned before surgery is called neoadjuvant therapy, while treatment given after surgery is called adjuvant therapy [7]. The therapeutic selectivity is based on the better conception of the biology and molecular genetics in the tumor progression used for the promising treatments [8], also, early diagnosis of the breast has a better prognosis [9]. Furthermore, understanding the behavior and biology of breast cancer is important to improve treatment strategies. However, there are some shortcomings to each of the current standard therapeutic procedures [10]. In this chapter; we are discussing available methods for breast cancer treatment.

Neo-Adjuvant Therapy and Adjuvant Therapy

The neo-adjuvant approach to breast cancer is used for locally advanced diseases. It is used before tumor removal that can allow breast-conserving surgery, rather than mastectomy [11]. Neo-adjuvant endocrine therapy or neoadjuvant chemotherapy before surgery is the best option for survival for most locally advanced breast cancer patients [12]. However, radiotherapy is not usually considered neoadjuvant therapy for breast cancer. Phase III trials are using radiation therapy following surgery and adjuvant chemotherapy [13]. Adjuvant therapy includes chemotherapy and endocrine therapy. In some cases, a combination of therapeutic approaches is more beneficial such as combined of both chemotherapy and endocrine therapy. The main role of adjuvant treatment is to improve overall survival. Patients with early breast cancer undergoing adjuvant therapy are clinically free of disease and may be cured by surgery alone. The goals of adjuvant treatment are to improve overall survival. The meta-analysis has shown that recurrence and mortality were reduced by the action of adjuvant hormone therapy and chemotherapy for breast cancer [14].

Surgery

Surgery or mastectomy is the procedure that involves the removal of the breast entirely or partially, depending on the stages of the breast cancer, size, location, and behavior of the tumor [15]. The early detection and removal of the cancerous tumor can help prevent cancer metastases to other parts of the body. There are different types of mastectomy including a simple mastectomy that removes the breast tissues but not all the lymph nodes. A modified radical mastectomy removes the entire breast including both the breast tissues and most of the axillary lymph nodes. Radical mastectomy is the most extensive type of mastectomy is recommended in breast cancer metastasis, it is referred to as total mastectomy and removal of all mammary tissue [16]. Partial mastectomy also called lumpectomy is the removal of the part of the breast tumor tissue and some normal tissue surrounding breast tissues [15]. Surgery is sometimes combined with other therapies to improve the efficacy of therapy response [17]. Most patients are given radiation therapy treatment that may help to destroy the residual cancer cells [18]. Axillary lymph node metastasis is considered an important factor when it comes to breast cancer prognosis [19]. Sentinel lymph node dissection (SLND) is the standard surgical procedure that is used for axillary node-negative patients who undergo surgery as the first line of their breast cancer treatment. However, many studies also demonstrated that the rate of sentinel lymph node (SLN) is considered 93–99% with a false negative rate of 4–5%. Furthermore, if the SLN is negative for metastases, then no further axillary surgery is required, while the patients with a positive SLN undergo axillary lymph node dissection. The axillary lymph node plays a critical role and affects survival in breast cancer, and is often used to guide the systemic therapy decisions [20].

Radiation Therapy

Radiation therapy is another standard strategy that involves the administration of a high dose of radiation to kill cancer cells. Ionizing Radiation (IR), like X-rays and gamma rays, are used for the treatment of tumors because they can pass through tissues. The ionizing radiation for tumors depends on the kind of radiation, amount of dose, and dose fractionation [21]. Although radiotherapy has an effective role in treating breast cancer, there are a large number of patients who are radio-resistant to treatment. Cancer cells are not killed immediately by ionizing radiation, and a substantial number of those patients need more time to recover. Thus, ionizing radiation alone is not effective enough for the treatment of breast cancer so combined radiation therapy with other anticancer treatments is considered to be more effective against cancer types that are radio-resistant [22]. In solid tumors, radiotherapy combination can have better results rather than

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