Review Article

Current Therapies and Novel Targets in Treatment of Breast Cancer

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A B S T R A C T

Breast cancer is the most common cause of cancer-related death among women worldwide, with case fatality rates highest in low-resource countries. Despite significant scientific advances in its management, most of the world faces resource constraints that limit the capacity to improve early detection, diagnosis, and treatment of the disease. There are different types of breast cancer, and different treatments that can work for each. Breast cancer is a highly complex disease with many treatment options including surgery, radiotherapy, hormonal therapy, biological therapy and chemotherapy. Optimizing standard treatment modalities for breast cancer has improved the outlook for women afflicted with it, but the fact that 40% still ultimately die from the disease highlights the need for new therapies. Remarkable advances in molecular immunology and biotechnology have created a unique opportunity for developing active vaccination strategies that engage the patient's own immune system in the fight against breast cancer. This article will review current drugs used in treatment of breast cancer and the novel targets which will be used safe and effective management of breast cancer.

Introduction

Breast cancer is a cancer that starts in the cells of breast.[1] Breast cancer is overwhelmingly a female disease, but about 1% of cases occur in men (around 300 per year in the UK).[2] After lung cancer, breast cancer is the second most common cancer in women worldwide and the fifth most common cause of cancer death.[3] In 2007, breast cancer caused 40,460 deaths worldwide and in 2008, an estimated 182,480 new cases of invasive breast cancer diagnosed among women, as well as an estimated 67,770 additional cases of *in situ* breast cancer.[4] Breast cancer is an urgent public health problem in high-resource regions and is becoming an increasingly urgent problem in lower source regions, where incidence rates have been increasing by up to 5% per year. Breast cancer like other cancers can be benign or malignant. Cells from malignant tumors can spread (metastasize) to other parts of the body. The most common are the bones, liver, lungs, and brain. The new tumor has the same kind of abnormal cells and the same name as the primary tumor.[5] Although the most important risk factor for the development of breast cancer is age, risk may be affected by age at menarche, first pregnancy, age at menopause, use of exogenous estrogens,susceptible gene BRCA1 and BRCA2 mutations, and family history.[6-8] Obesity and heavy drinking also significantly increases the risk.[9,10] Although early detection and improved treatment modalities over the years have increased survival rates, extensive efforts have been directed at improving outcomes with more targeted therapies.

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Treatment of breast cancer

Cancer treatment is either local therapy or systemic therapy

Local therapy Surgery and radiation therapy are local treatments

They remove or destroy cancer in the breast. When breast cancer has spread to other parts of the body, local therapy may be used to control the disease in those specific areas.[11]

a) Surgery
Surgery is the most common treatment for breast cancer. There are several types of surgery.

- Breast-sparing surgery: An operation to remove the cancer but not the breast is breast-sparing surgery. It is also called breast-conserving surgery, lumpectomy, segmental mastectomy, and partial mastectomy. Sometimes an excisional biopsy serves as a lumpectomy because the surgeon removes the whole lump. The surgeon often removes the underarm lymph nodes as well. A separate incision is made. This procedure is called an axillary lymph node dissection. It shows whether cancer cells have entered the lymphatic system. After breast-sparing surgery, most women receive radiation therapy to the breast. This treatment destroys cancer cells that may remain in the breast.[12]

- Mastectomy: An operation to remove the breast (or as much of the breast tissue as possible) is a mastectomy. In total (simple) mastectomy, the surgeon removes the whole breast. Some lymph nodes under the arm may also be removed. In modified radical mastectomy, the surgeon removes the whole breast, and most or all of the lymph nodes under the arm. Often, the lining over the chest muscles is removed. A small chest muscles also may be taken out to make it easier to remove the lymph nodes.[13,14]

b) Radiation therapy
X-Ray Radiation therapy (XRT) is used to "sterilize" the remaining breast. XRT destroys cells by fracturing their DNA sequence through free radical creation and release. Complications associated include fatigue, breast erythema and edema, ipsilateral extremity edema long term (5-17%), and rib fractures.[15,16]

Systemic therapy

Hormone therapy, biological therapy and chemotherapy are systemic treatments. These therapies can be initiated before surgery, as in neoadjuvant therapies, or after surgery, as in adjuvant therapies. They enter the bloodstream and destroy or control cancer throughout the body.

Anti-hormonal medication

Estrogen is the major growth promoter for the breast cancer cells. In most cases cancer cells have receptors that allow circulating estrogen to attach to the tumor cell, providing food for growth. It is desirable to have this receptor, which makes the tumor estrogen receptor(ER)-positive and/or progestrone receptor (PR)-positive.[17] This type of tumor cell is potentially responsive to anti-hormonal systemic treatment [Table 1]. If a tumor is ER and PR negative, then it is unlikely that the anti-hormonal drugs will be used. Often, chemotherapy alone is suggested as systemic treatment. If a tumor is ER or PR positive, often both chemotherapy and anti-hormonal therapy are used, depending on the stage of the cancer. Sequential endocrine therapy continues as long as the patient remains hormone sensitive. Once hormone-resistant disease develops, chemotherapy is the current alternative.

Table 1: Hormonal therapies for breast cancer[18,30]

Class	Indication	Dosage/Route	Common adverse drug reactions
Selective ER Modulators			
Tamoxifen (Novaldex)	MBC In men and women, adjuvant therapy in axillary node-postive and node- negative breast cancer following surgical resection	20 mg PO qd	Hot flashes, DVT, PE, endometrial hyperplasia, uterine polyps, endometrial cancer, uterine sarcoma, triglyceride elevation, skin rash, visual disturbances,
Toremifene (Fareston)	MBC in postmenopausal women with ER+ tumors or ER unknown tumors	60 mg PO qd	myelosuppression, Hot flashes, sweating, menustral irregularity, tumor flare, anorexia, myelosuppression, skin rash, alopecia, peripheral edema
Selective Non steroidal Aromatase Inhibitors-Postmenopausal women Only			
Anastrozole (Arimidex)	MBC for postmenopausal women with ER+ or ER unknown tumors First line or second line with progression on tamoxifen	1 mg PO qd	Asthenia, N&V,Hot flashes, skin rash, arthralgia, diarrhea, headache, peripheral edema, Flu like syndrome
Letrozole (Femara)	Adjuvant treatment of postmenopausal women with hormone receptor positive early stage breast cancer	2.5 mg PO qd	
Selective steroidal Aromatase Inactivator-For Postmenopausal women only			
Exemstane (Aromasin)	Treatment of advanced breast cancer in postmenopausal women whose disease has progressed following tamoxifen therapy	25 mg PO qd	Hot flashes, fatigue, nausea, headache, arthralgia, diarrhea
Antiestrogen			
Fulvestrant (Faslodex)	MBC treatment of postmenopausal women with ER+ tumors advanced disease with progression following antiestrogen therapy	250 mg IM monthly	Asthenia, N&V, Hot flashes, headache, injection site reactions, back pain, arthralgia, Flu like syndrome, dry scaling rash
Progestin			
Megestrol Acetate (Megace)	Breast cancer	25 mg PO qid	Weight gain, thromboembolic events, N&V, breakthrough menustral bleeding, tumor flare, hyperglycemia,,hot flashes
Androgen			
Fluoxymestrone (Halotestin)	Inoperable breast carcinoma	10-40 mg in divided doses for 1-3 months	Amenorrhoea, edema, N&V, hypercalcemia, leukopenia, hepatic necrosis, hypersensitivity reactions
Estrogen			
Ethinyl estradiol (Estinyl)	Inoperable progressing breast cancer	1 mg PO tid	Photosesitivity, thromboembolic complications, disturbance of vision or speech, mental depression, unusual bleeding
LHRH agonist			
Goserelin (Zoladex)	Advanced breast cancer	3.6 mg SC every 28 days or 10.8 mg depot every 3 months	Bone pain, headache, edema, rash, N&V bleeding, injection site pain

ER - Estrogen receptor; MBC - Metastatic breast cancer; PO - By mouth; qd-Every day; DVT - Deep venous thrombosis; PE - Pulmonary emboli; NandV - Nausea and vomiting; IM - Intramuscularly; qid-Four times a day; tid-Three times a day; LHRH - Lutenising hormone-releasing hormone; SC - Subcutaneously

a) *Selective estrogen receptor modulators*

Tamoxifen (NovaldexR) is one of the oldest used SERMs. It inhibits the growth of breast tumors by competitive antagonism of estrogen at its receptor site.[18,19] It also exhibits partial estrogen-agonist effects. Tamoxifen is normally taken orally for five years, beyond which there seems to be little additional benefit. As adjuvant therapy postoperatively it is the current standard first-line agent for patients with early, ER positive and/or PR positive breast cancer. It is also indicated as adjuvant therapy in patients with metastatic disease. In the chemoprevention setting, tamoxifen is the only available endocrine option for women at high risk of breast cancer but, given that these are healthy subjects, it is associated with an unacceptable rate of adverse events.[20] Toremifene (FarestonR), another antiestrogen closely related to tamoxifen may be an option for postmenopausal women with metastatic breast cancer. Newer SERMs, such as raloxifene (EvistaR), were initially approved to lower the risk of osteoporosis.[21] Raloxifene's anti-cancer and chemopreventive effects are currently being investigated in the STAR (Study of Tamoxifen and Raloxifene) trial.[20]

b) *Progestins*

PR-positive advanced breast tumors can respond to the use of synthetic progesterone-like drugs such as megesterol acetate (MegaceR). Megestrol acetate was also shown to reduce the frequency of hot flushes in postmenopausal breast cancer patients.[22] Progestins are usually restricted to second or third line therapies following aromatase inhibitors and/or antiestrogens.

c) *Lutenising Hormone Releasing Hormone agonists*

LHRH analogs such as goserelin (ZoladexR) and luprolide (LupronR) are a group of drugs that suppress ovarian estrogen production down to postmenopausal levels, essentially inducing a potentially reversible medical ovarian ablation. They are most effective in ER-positive early breast cancer in premenopausal women.[23]

d) *Aromatase Inhibitors*

In postmenopausal women, estrogen synthesis occurs in non-ovarian peripheral tissues. This mainly follows the route of conversion by aromatase, of the androgenic substrates androstenedione and testosterone to estrone and estradiol in the adrenal glands and adipose tissue. AIs are of no value in premenopausal patients where the ovaries are the primary sites of estrogen production.[24] There are two main structural types of aromatase inhibitor:1) steroidal, substrate analogs such as 4-hydroxyandrostenedione (formestane) and exemestane, and 2) reversible nonsteroidal imidazole-base inhibitors (e.g. anastrozole (ArimidexR) and letrozole (FemaraR).[25] These are known as type I and type II inhibitors, respectively. Use of third-generation AIs lowers total body aromatization and plasma estradiol levels by more than 95%.[26]

e) *Estrogen receptor antagonists*

An important addition to the armamentarium of endocrine therapies is the selective estrogen-receptor antagonist fulvestrant (FaslodexR), also termed "an estrogen receptor down-regulator.[27] As a steroidal analog of 17β-estradiol, fulvestrant has a chemical structure that is similar to that of estradiol, but distinct from tamoxifen and other nonsteroidal hormonal agents. Both tamoxifen and fulvestrant competitively inhibit the binding of estradiol to the ER. In contrast to tamoxifen, fulvestrant has no agonist effect and downregulates the expression of the ER. With estradiol as the comparator, fulvestrant's ER binding affinity (0.89) is greater than tamoxifen's (0.025).[28,29]

Biological therapy

Human epidermal growth factor receptor 2 (HER2) has been found to be an important prognostic and predictive marker of treatment response in women with breast cancer in the adjuvant setting and advanced disease. The HER2 gene is amplified and the HER2 protein is overexpressed in 20% to 25% of breast cancers with resulting poor prognosis and shortened overall survival (OS).[31] The HER2 gene, also known as HER2/neu is located on chromosome 17q and belongs to the human epithelial receptor (HER) family of genes. It encodes a 185 kDa transmembrane tyrosine kinase growth factor receptor, which mediates signaling for cell proliferation and survival).[32] HER2 gene amplification and resultant protein overexpression are associated with a more aggressive clinical course. Several murine monoclonal antibodies against the extracellular domain of the HER2 protein have been found to inhibit proliferation of cells overexpressing HER2.[33] However, to minimize immunogenicity, the antigen-binding region of one of the more effective antibodies was fused to the framework region of the human IgG leading to trastuzumab. Trastuzumab (Herceptin, Genentech Inc., South San Francisco, California, U.S.A.) is a humanized monoclonal antibody that binds to the HER2. It was approved in 1998 by the U.S. Food and Drug Administration (FDA) for the treatment of HER2-positive metastatic breast cancer (MBC) in the first-line setting in combination with paclitaxel, or as monotherapy for patients who had received at least one prior chemotherapy regimen for HER2-positive MBC. Trastuzumab is now predominantly used in combination with chemotherapy in the first-line setting of metastatic disease due to its clear advantage in improving clinical outcome.[34,35]

Chemotherapy

They are used to kill circulating cancer cells that could grow *in vital* organs, causing metastatic cancer (cancer which has spread beyond the breast).Women with ER+ or PR+ tumors, symptomatic visceral metastasis, or hormone refractory disease should receive chemotherapy. Table 2: Identifies preferred first-line single agents for breast cancer. Other active agents include cisplatin, carboplatin, paclitaxel protein-bound particles for injectable suspension, etoposide, vinblastine, and fluorouracil by continuous infusion. Adjuvant combination chemotherapy offers higher response rates and longer time to disease progression. Table 3: Lists first-line combination regimens e.g., cyclophosphamide, doxorubicin, and fluorouracil (FAC/CAF); fluorouracil, epirubicin, and cyclophosphamide (FEC); doxorubicin and cyclophosphamide (AC); epirubicin and cyclophosphamide (EC); doxorubicin in combination with docetaxel or paclitaxel (AT); and cyclophosphamide, methotrexate, and fluorouracil (CMF).[36-39]

Novel targets in therapy of breast cancer

Anti-epidermal growth factor receptor strategies for advanced breast cancer

The ErbB family of receptors belong to the type I superfamily of receptor tyrosine kinases. Four members of this family have been

Table 2: Single chemotherapy agents for breast cancer[30,37]

Chemotherapy	Agent (mg/m^2)	Dosage	Schedule	Interval
Doxorubicin (Adriamycin)	60-75	IV	D1	Every 21 days
Doxorubicin (Adriamycin)	20	IV	Weekly	
Epirubicin (Elience)	60-90	IV	D1	Every 21 days
Pegylated liposomal Doxorubicin	60	IV	D1	Every 28 days
Paclitaxel (Taxol)	175	IV	D1	Every 21 days
Paclitaxel (Taxol)	80	IV	Weekly	
Paclitaxel protein bound Particles for injectables Suspension(abraxane)	260	IV	D1	Every 21 days
Docetaxel (Taxotere)	60-100	IV	D1	Every 21 days
Docetaxel (Taxotere)	40	IV	Weekly	For 6 weeks followed by 2-week rest, then repeated
Vinorelbine	25	IV	Weekly	Every 21 days
Capecitabine (Xeloda)	1000-1250	PO	Weekly	Every 28 days
Gemictabine (Gemzar)	800-1200	IV	Twice daily D1, D8 & D15	

IV - Intravenous; D1 - Day1; PO - by mouth; D8 - Day 8; D15 - Day15

identified: Epidermal growth factor receptor (EGFR) or ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4. They are expressed in a variety of tissues including epithelial, mesenchymal, and neural origin, where they exert effects on development, cellular proliferation, and differentiation.[40] Structurally, all ErbB receptors share in common an extracellular domain, a membrane-spanning domain, and an intracellular domain that encompasses the tyrosine kinase activity. Through a complex network of downstream cascades, their dysregulation confers poorer prognosis in breast and other solid tumors that overexpress them.[41-44] The ErbB family of receptor tyrosine kinases has quickly become one of the most important signaling pathways found in human breast cancer.[45] Its dysregulation leads to a more aggressive cancer phenotype and its inhibition can act as a highly effective therapeutic strategy. Till date, there are a number of small molecule tyrosine kinase inhibitors with documented activity in ErbB2-overexpressing breast cancer that are being tested for improved efficacy in the treatment of breast cancer.[46] The small molecule inhibitor with the most clinical data is a dual ErbB1/2 inhibitor, lapatinib with which multiple clinical trials are still on going. Table 4 represent ErbB Family and Their Small Molecule Inhibitors

Farnesyl transferase inhibitors

Ras proteins belong to the small guanine triphosphate-binding protein (G protein) superfamily that is widely distributed in mammalian cells.[53,54] G proteins regulate a wide variety of cellular functions, including gene expression in normal cell growth and differentiation (Ras), cytoskeletal reorganization and gene expression (Rho), vesicle trafficking (Rab and Sar1/Arf), nucleocytoplasmic transport (Ran), and microtubule organization (Ran). Three classes of isoprenyltransferase enzymes have been identified in mammalian cells, including protein farnesyl transferase (FTase), type I protein geranylgeranyltransferase (GGTase-I), and type II protein geranylgeranyltransferase (GGTase-II). FTase catalyzes farnesylation of proteins in which X is methionine, serine, alanine, glutamine, or cysteine (e.g., Ras, Lamin B, Rho B) and GGTase-I catalyzes geranylgeranylation of proteins in which X is leucine, isoleucine, or phenylalanine (e.g., Rho, Rap, and Rac). GGTase-II catalyzes the geranylgeranylation of sequences CXC, CCX, or XXCC (e.g., Rab proteins). Both FTase and/or GGTase have been considered as potential therapeutic targets.[55-59] At least three different strategies have been developed to target the aberrant Ras/G protein pathway in cancers: (i) blocking upstream activation of Ras at the cell surface receptors (such as ER, HER2/neu, EGFR, or other receptor tyrosine kinases); (ii) targeting Ras itself by inhibiting either Ras gene expression (e.g., antisense molecules) or interrupting protein processing (e.g., farnesyl transferase or geranylgeranyl transferase inhibitors); and (iii) inhibiting downstream effector pathways (e.g., Raf kinase or MEK inhibitors.[60-63] Most preclinical and clinical studies to date have been focused on inhibiting Ras/G protein prenylation with farnesyl transferase inhibitors (FTIs).[64-67] FTI s have been classified into three subclasses, including (i) farnesyl pyrophosphate analogs (nonpeptidomimetics), which compete with the isoprenoid substrates for FTase, (ii) peptidomimetic inhibitors, which mimic the structure of CAAX portion of Ras and compete with Ras for FTase and (iii) bisubstrate analogs, which combine the properties of both. Two oral FTIs that have been most extensively studied in clinical trials ranging from phase I to phase III trials, including the nonpeptidomimetic agents tipifarnib (R115777, Zarnestra™; Johnson and Johnson Pharmaceutical Research and Development, U.S.A.) and lonafarnib (SCH66336, Sarasarw; Schering-Plough, Inc., Kenilworth, New Jersey, U.S.A.).[68] However, only tipifarnib has been evaluated in breast cancer, both as a single agent[69] in combination with hormonal therapy[70] and chemotherapy.[71]

The epothilones

The epothilones, a promising new class of microtubule-stabilizing compounds, have commanded attention recently, as their mechanisms of action are similar to those of the taxanes, yet they have the potential to evade the known mechanisms of taxane resistance This feature of the epothilones makes them valuable agents for the treatment of patients with taxane-resistant disease, an increasingly large population of patients with recurrent breast cancer.[72] Modifications of the structure of naturally occurring epothilones have yielded multiple biologically active analogues with varying activity and toxicity profiles.[73-76] The three principal epothilone analogues under active development in breast cancer are ixabepilone (BMS-247550, aza-epothilone), patupilone (EPO906,

Table 3: Common adjuvant chemotherapy regimens[36-39]

	Regimen	Dosage (mg/m²)	Route	Schedule	Interval	Cycles
FAC	5-Fluorouracil	500-60	IV	D1 & D8	Every 21-28 days	4-6
	Doxorubicin	50-60	IV	D1		
	Cyclophosphamide	500-600	IV	DI		
CAF	Cyclophosphamide	100	PO	D1 & D14	Every 28 days	6
	Doxorubicin	30	IV	D1 & D8		
	5-Fluorouracil	500	IV	D1 & D8		
AC	Doxorubicin	60	IV	DI	Every 21 days	4
	Cyclophosphamide	600	IV	DI		
AC→T	Doxorubicin	60	IV	D1	Every 21 days	4
	Cyclophosphamide	600	IV	D1		
	Followed by paclitaxel	175-225	IV	D1	Every 21 days	4
4 Cycles of AC followed by 4 cycles of T						
Dose-dense	Doxorubicin	60	IV	D1	Every 21days	4
AC→T	Cyclophosphamide	600	IV	D1	Every 14 days	
	Followed by Paclitaxel	175	IV	D1		4
	Filgrastim	5	SC	D3-D10	With each weekly cycle	
4 Cycles of AC followed by 4 cycles of T 13%of patients require pRBC transfusion						
Oral CMF	CyclophosphamideMethotrexate	100	PO	D1& D14	Every 28 days	6
	5-Fluorouracil	40	IV	D1& D8		
		600	IV	D1& D8		
TAC	Docetaxel	75	IV	DI	Every 21 days	6
	Doxorubicin	50	IV	DI		
	Cyclophosphamide	500	IV	DI		
A followed by CMF	Doxorubicin followed by cyclophophamide	75	IV	D1	Every 21 days	4
	Methotrexate	600	IV	D1		
	5-Fluorouracil	40	IV	D1	Every 21 days	8
		600	IV	D1		
CEF	CyclophosphamideEpirubicin	75	PO	D1& D14	Every 28 days	6
	5-Fluorouracil with prophylactic antibiotics	60	IV	D1& D8		
		500	IV	D1& D8		
FEC100	5-Fluorouracil Epirubicin	500	IV	D1	Every 21 days	6
	Cyclophosphamide	100	IV	D1		
		500	IV	D1		
IV CMF	Cyclophosphamide Methotrexate	500	IV	D1 & D8	Every 28 days	6
	5-Fluorouracil	40	IV	D1 & D8		
		600	IV			

IV - Intravenous; D - day; PO - by mouth; SC - Subcutaneous

epothilone B), and KOS-862 (epothilone D).[77,78] The development of two other analogues, ZK-EPO and BMS-310705 (a water-soluble epothilone B analogue) has been put on hold.[79] Ixabepilone, patupilone, and KOS-862 all have broad-spectrum antitumor activity in cell culture and xenograft models.[73,76] Each of the three analogues exhibits more potent tubulin-binding than paclitaxel, with tubulin polymerization induced and stabilized at lower concentrations. In addition, these epothilone analogues are generally 5 to 25 times more potent than paclitaxel in inhibiting cell growth in cultures. Furthermore, unlike the taxanes, the epothilones are cytotoxic against multi-drug resistant cell lines and against cells containing tubulin mutations that result in taxane-resistance.[80]

Nab-paclitaxel: Reducing toxicity using albumin-bound particles as the carrier for paclitaxel

The taxanes, paclitaxel and docetaxel, are some of the most effective chemotherapeutic agents, and have an important role in the treatment of breast cancer. Because taxanes are not soluble in aqueous solution, they require a vehicle to solubilize them in an injectable form. Polyoxyethylated castor oil (Cremophor EL; CrEL) and ethanol were used as vehicles for the first clinically available formulation of paclitaxel (solvent-based paclitaxel). Solvent-based paclitaxel was found to be associated with severe hypersensitivity reactions in reports of adverse drug reactions during phase-1 trials.[81] Nonclinical and clinical evidence suggests that polyoxyethylated castor oil may contribute to these hypersensitivity reactions from solvent-based paclitaxel.[82,83] The reformulation of paclitaxel with albumin circumvents solvent-associated toxicity and utilizes the natural carrier role of albumin in the human circulation.[84] Paclitaxel is homogenized with albumin using 130-nanometer albumin-bound (nab) technology to produce a colloidal suspension for intravenous infusion (nab-paclitaxel).[85] A nonclinical study of nab-paclitaxel and solvent-based paclitaxel compared mortality data at the 30 mg/kg/day doses of nab-paclitaxel and solvent-based paclitaxel.[86] Toxicity was significantly less with nab-paclitaxel (P ¼ 0.0017, Analysis of Variance). The maximum tolerated doses were 30 mg/kg/day for nab-paclitaxel and 13.4 mg/kg/day, which were considered to be equitoxic doses (4% for both). Combinations of nab-paclitaxel with chemotherapeutic agents and biologic agents have been examined in phase II trials.[87]

Table 4: ErbB family and their small molecule inhibitors[46-52]

Target Receptor	Ligand(s)	Inhibitor	Phase of clinical development in Breast Cancer
ErbB1	EGFR	AG1478/PD158780/EKB569	I
	TGF-alpha	Gefitinib/Erlotinib	II
	Amphiregulin	CI1033(Canertinib)	II
	Epiregulin	Lapatinib(GW572016)	III
	Betacellulin		
	HB-EGF		
ErbB2	-	AG1478/PD158780/EKB569	I
		Lapatinib(GW572016)	III
ErbB3	Epiregulin	-	
	Neuregulin1/2		
ErbB4	Neuregulin1/2	-	II
	CI1033(Canertinib)		
	Neuregulin3/4		I
	PD158780		
	Epiregulin		
	Betacellulin		
	HB-EGF		
ErbB2	-	AG1478/PD158780/EKB569	I
		Lapatinib(GW572016)	III
ErbB3	Epiregulin	-	
	Neuregulin1/2		
ErbB4	Neuregulin1/2	-	II
	CI1033(Canertinib)		
	Neuregulin3/4		I
	PD158780		
	Epiregulin		
	Betacellulin		
	HB-EGF		

Antiangiogenic agents in breast cancer

Angiogenesis represents a complex mechanism of finely regulated mediators that act to promote new blood vessel growth and migration.[88] In 1971, Folkman described the association between angiogenesis and the malignant potential of solid neoplasms, and proposed that without neovascularization, tumors would reach a maximum diameter of 2 to 3 mm (the maximum distance for the adequate diffusion of oxygen), and then enter a dormant state.[89] With appropriate stimulus (e.g., hypoxia, metabolic stress, and inflammation) the balance is "tipped" in favor of angiogenesis, and the switch promoting new vessel growth and recruitment is activated. Hypoxia is the characteristic event, which leads to the expression of hypoxia induced factor- 1a (HIF-1a), triggering a cascade of events that culminates in the transcription of mRNA and the resultant increased expression of VEGF.[90] Upon binding to its receptors, VEGF activates crucial signaling pathways leading to cell proliferation, increased vasopermeability, inhibition of apoptosis, and ultimately angiogenesis. Hypoxia is not the only stimulus for VEGF expression, and increased transcription of VEGF has been associated with a variety oncogenes, including mutant ras, erbB-2/HER2, activated epidermal growth factor receptor (EGFR). Many solid tumors produce VEGF as means of promoting pathologic angiogenesis, and up-regulation of VGEF mRNA has been found in the vast majority of human malignancies, including breast cancer.[91,92] Bevacizumab has been shown to effectively bind the soluble VEGF-A ligand, preventing binding to its receptors (Flt- 1 and KDR/Flk-1), and essentially disrupting the initial signal in the angiogenic cascade. Many approaches are still under investigation, the most studied and successful to date involves the development of a monoclonal antibody directed against the VEGF-A isoform, the most predominant and active ligand in this pathway. Bevacizumab (Avastinw, Genentech, San Francisco, CA) is currently the only FDA approved monoclonal antibody aimed at specifically inhibiting angiogenesis in solid tumors. While bevacizumab is currently only approved for use with bolus IFL (irinotecan, 5-FU and leucovorin) in first-line therapy for metastatic colorectal cancer[93] it has shown potential in early trials investigating its use in nonsmall lung cancer,[94] renal cell carcinoma,[95] and breast cancer[96]

Epigenetic regulation as a new target for breast cancer therapy

Epigenetics is a process by which gene expression may be modulated without an alteration in the primary nucleotide sequence of a gene.[97] Epigenetic regulation is critical in normal growth and development and provides a layer of transcriptional control of gene expression. Stability of DNA structure requires faithful replication of DNA, and alterations may lead to abnormal processes, such as autoimmune disease, genetic disorders, and cancer. A prominent epigenetic alteration is DNA-methylation in the promoter region of the gene that prevents the gene to be expressed. Epigenetic changes may be inherited or result from environmental exposures. Epigenetic changes can be implicated both in cancer initiation and progression. Because epigenetic changes may be reversible, they represent an active area for new drug investigation and are promising targets for cancer therapy.[98]

DNA methylation

In replicating DNA (i.e., in dividing cells), enzymes called DNA

methyltransferases (DNMTs) add a methyl group to the cytosine ring to form methyl cytosine. This modification takes place only on acytosine that precedes a guanosine in the DNA sequence, called the CpG dinucleotide. During evolution, the number of CpG dinucleotides in the genome has been depleted because of mutations, resulting in only a small number of such sites compared to a mathematically expected number. However, several small regions of DNA contain the expected number of CpG dinucleotides, the so-called CpG islands. CpG islands are generally present at the promoter region of most genes. CpG dinucleotides that are not in CpG islands are usually methylated, resulting in suppression of transcription. In contrast, most CpG dinucleotides in CpG islands in gene promoter regions are unmethylated and allow for active gene transcription.[97] In cancer cells, CpG islands that are normally unmethylated may become methylated, resulting in silencing of important genes, such as inactivation of tumor suppressor genes. At the same time, CpG dinucleotides in other regions may become unmethylated, leading to diminished transcriptional repression of normally silenced genes such as oncogenes. DNA methylation is mediated by several proteins. As noted, DNMTs add methyl groups to the cytosines in CpG dinucleotides. Three active DNMTs have been recognized in humans and are designated DNMT1, DNMT3a, and DNMT3b. Each DNMT may have a specific role in the methylation process, or may act in association with another methyltransferase. DNMTs are also responsible for the recruitment of histone deacetylases (HDACs) to the sites of gene promoters, and may bind to other proteins with a goal of maintaining a repressed transcriptional status. Several DNMT inhibitors are under investigation for cancer treatment.[99] The identification of methylated genes is also under investigation. Changes in gene methylation or histone acetylation may serve as biomarkers of cancer risk, assist in cancer detection, provide molecular staging, or predict prognosis or response to treatment.[100] Importantly, epigenetic changes represent an exciting target for therapy.

Tumor vaccines for breast cancer

The goal of cancer vaccines and immunotherapies is to train the immune system to recognize cancer cells and destroy them.Immune responses play a dynamic role in the development of cancers, from immuno surveillance to immune escape; from *in situ* immune dysregulation to metastatic spread. The systematic identification and targeting of molecules involved in the immune response has led to a wide variety of potential immunotherapeutic targets for the treatment of breast cancer.[101] To date, most vaccine strategies have focused on immune activation such as antigenic delivery, TLR activation by CpGs and adjuvant, and cytokine stimulation. However, the identification of immune regulatory pathways, such as B7-H1, B7-H4, CTLA-4, IDO, and regulatory *t*-cells has demonstrated that inhibition of immune regulation will be critical to establish effective anti-tumor immunity.[102] The successful development of breast cancer vaccines will require combinatorial therapies that target both breast-cancer specific immune activation and inhibition of immune tolerance.

Antigen based vaccine

The ideal breast cancer vaccine would induce broadly reactive immunity to multiple types of breast cancer without causing clinically significant autoimmunity and, most important, be clinically effective. One approach to minimize autoimmunity and enhance specificity of vaccines is to target them to specific protein antigens that are over expressed on the tumor cells but that have limited distribution in normal tissue. Many breast cancer tumor antigens are also expressed on tumor cells in other epithelial-derived cancers, such as ovarian cancer and colon cancer, and have been targeted in early-phase clinical trials in breast cancer and other solid tumors. In addition to MUC-1, HER2/neu, and telomerase, target antigens include CEA[103,104] cyp1B1,[105] surviving[106,107]and others

Cellular-based vaccines

Vaccines based on whole autologous or allogeneic tumor cells have been combined with strong adjuvants or cytokines, since tumor cells themselves generally stimulate poor antigen presentation.[108] Both autologous tumor cells[109-111] and allogeneic cell lines[112-114] have been used in clinical trials in breast cancer, with isolated clinical responses reported. Whole tumor cells have also been fused with dendritic cells. In murine models, GM-CSF was the most potent cytokine adjuvant for vaccination and GM-CSF-secreting autologous and allogeneic vaccines are currently being evaluated in clinical trials in breast cancer.

Conclusion

Considerable progress has been made in the understanding of the molecular basis of breast cancer. Many endocrine agents proved to be beneficial as adjuvants and in advanced hormone-responsive breast cancer. These include selective estrogen receptor modulators, third-generation aromatase inhibitors, progestins, and LHRH analogs. Despite this, cytotoxic chemotherapy is still the mainstay of treatment especially in the metastatic setting. Although still under trial, novel targeted drug therapies including; anti-epidermal growth factor receptor strategies, farnesyl transferase inhibitors, epothiolones, antiangiogenic agents, epigenic regulation and tumor vaccines may give a new horizon for future management of breast cancer.

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