ROLE OF RECEPTORS IN BREAST CANCER

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ABSTRACT
Breast cancer is a major concern and one of the leading causes of cancer-related death worldwide. Breast cancer, like many other types of cancer, is a complex heterogeneous disease controlled by a multitude of genetic and epigenetic alterations. The hormonal status of ER and PR, HER2/neu helps in identifying the non aggressive behavior and patients likely to achieve benefit from endocrine therapy. ER positive primary malignancies of breasts show good response to hormone therapy. Triple-negative (ER-negative, PR-negative, HER2/neu negative) breast cancer has distinct clinical and pathologic features and it has typically high grade, relatively poor prognosis, aggressive behavior and lack of targeted therapies leaving chemotherapy as the mainstay of treatment. Understanding the frequency of occurrence of specific breast cancer subtypes and associated risk factors may elucidate the breast cancer prevention, screening and treatment strategies.

INTRODUCTION
Breast cancer is a serious life threatening condition observed in women worldwide. It ranks second (after lung cancer) as a cause of cancer death in women. In the central region of India, 50 to 70% of breast cancer patients present in an advanced stage (ICMR, 1989). In US, from 1975 through 2003, 394,891 invasive and 59,837 in situ breast cancer cases were diagnosed in women (Hausauer et al., 2007; Jemal et al., 2007). It is one of the most frequently malignant tumors diagnosed in women worldwide (Parkin et al., 2000). It accounts for 22% of all female cancers, which is more than twice the prevalence of cancer in women at any other site (Parkin et al., 2000). Presently, 75,000 new cases are reported annually and account for 19-34% of all cancer cases among women nationally (ICMR 1989; Badwe et al., 1990; Murthy et al., 1990; ICMR 2001; Siddqui et al., 2001; Saxena et al., 2005). The life time risk of developing breast cancer in women is 13% and 210,000 new cases are diagnosed each year in the United States (Dowsett et al., 2007).

Estrogen & Progesterone Receptor
Determination of hormonal status is an important primary assessment at the time of a breast cancer diagnosis. Significant prognostic and therapeutic implication of estrogen receptor in breast cancer patients is now recognized. Expression of estrogen receptor is present in about 70% of breast cancer cases (Gown et al., 2008). Estrogen receptor (ER) status is an important predictive and prognostic factor in breast cancer (Rastelli et al., 2008).

ERs expression has been considered to be present in two-thirds of breast cancers (Alfred et al., 1998) but more recent studies suggest that its incidence may be closer to 70% (Nadji et al., 2005). ER status is strongly influenced by tumor grade and histology (Anderson et al., 2002). ER expressed in 70% to 95% of invasive lobular carcinomas. ER-negative breast cancers are more aggressive (Sheikh et al., 1994) through a still unknown mechanism (Rastelli et al., 2008). Epidemiologic studies have shown that the percentage of ER positive breast cancers has been increasing over time through an unknown mechanism.

In classical mechanism, binding of estrogen to estrogen receptor, resulting ligand-receptor complex bind to DNA at estrogen response elements (ERE) in the promoters of target gene (Lone et al., 2004).

In non classical mechanism, the estrogen-ER complex can promote transcription via activator protein (AP)-1 and specificity protein (SP)-1 complexes (Heldring et al., 2007). These proteins regulate the normal biological processes (Sharrocks et al., 1997) and have oncogenic & tumor suppressive activity (Zhou et al., 1998; Ma et al., 2003). In several studies differential expression of genes (e.g. GATA3, TFF1) have shown in ERa (+) and ERb (-) breast cancers (West et al., 2001; Mehra et al., 2005).

Estrogen stimulates proliferation of breast cancer cells, in large part via estrogen receptors (ERs; members of the superfamily of nuclear receptors) (Simpson et al., 2003). There are two main subtypes of ERs: ERα and ERβ. Both the ERs functionally act as transcription factors to initiate target gene expression (Heldring et al., 2007). Clinically relevant studies have established that naturally occurring estrogens play a critical role in the initiation, progression and maintenance of breast cancers. Under physiological conditions, estrogens are vital for the normal development, growth control, differentiation and function of breast (Shekhar et al., 2001).

Risk factors
In epidemiologic studies, percentage of ER positive breast cancers has been increasing over time (Pujol et al., 1994; Li et al., 2003). The reason for this increase is unclear, but it may be due to environmental factors (Darbre et al., 2006; Salehi et al., 2008). Lymph-node status and histological grade of the primary tumor (Bloom et al., 2007; Jemal et al., 2007).
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1957) are established as good predictors of disease-free interval but the role of ER and PR in predicting the likelihood of recurrence is more controversial. Factors used to distinguish between positive and negative results (Clarke et al., 1983) the length of follow-up (Raemaekers et al., 1985), and subsequent therapy after primary mastectomy (Raemaekers et al., 1987) have all been shown to influence the performance of ER and PR as predictors of recurrence or non-recurrence. Many risk factors are responsible for ER positive breast cancers. Ethnicity appears to be a factor in the occurrence of ER positive breast cancers (Gapsnur et al., 1996; Joslyn et al., 2002; Chu et al., 2002). ER+ breast cancers are associated with age at diagnosis and the use of postmenopausal hormone replacement therapy, but not with family history, benign breast disease, alcohol use or height (Colditz et al., 2004).

The risk of breast cancer is increased in postmenopausal women with high estrogen levels as well as in women with high androgen levels (Hankinson et al., 1998; Cauley et al., 1999). Many studies have reported that androgens can induce proliferative changes in breast tissue, and administration of both estrogen and androgens can induce tumor formation in animal model (Wong et al., 2001; Xie et al., 2001).

Prognosis

The data concerning progesterone receptor (PR) are not as clear, but ER-negative tumors with PR are thought to have an intermediate response rate compared with ER- and PR-positive tumors (Osborne et al., 1980). Estrogen (ER) and progesterone (PR) is expressed in 70% to 95% of invasive lobular carcinomas and 70% to 80% of invasive ductal carcinomas and PR is expressed in 60% to 70% of invasive breast carcinomas (Garau et al., 1996; Zafrani et al., 2000). Expression of ER or PR generally is associated with a better outcome. Survival and response to hormone therapy are most favorable among women with tumors positive for both ER and PR, least favorable for tumors negative for both (Heuson et al., 1977; Allegra et al., 1980; Campbell et al., 1981). Patients with ER-positive/PR-positive tumors have a better prognosis than patients with ER-positive/PR-negative tumors and these have a better prognosis than patients with ER-negative/PR-negative tumors (Gown et al., 2008). ER positivity also is prognostic of delayed recurrence in primary breast cancer (Knight et al., 1977).

ERα status is very much essential for endocrine therapy with anti-estrogens. Approximately half of all ERα (+) patients fail to respond to anti-estrogen therapy has been observed (Oh et al., 2006). Other clinical study suggested ERα (+) status correlates with improved prognosis, lower risk of relapse and better overall survival (Ring et al., 2006).

Metastatic breast cancer with ER+ tumors is more likely to respond to endocrine therapy (McGuire et al., 1975). Response rate of estrogen receptor (ER) positive tumors to hormonal therapy has been reported in approximately 50% to 75%, while ER-negative tumors have a less than 10% chance of response (Osborne et al., 1980; Wittliff et al., 1984).

Estrogen receptor positive tumors patients have an improved overall prognosis and more likely to respond to antiestrogen therapy for advanced disease (Block et al., 1978; Gapinski et al., 1980; Manin et al., 1980; Leake et al., 1981). Patients with estrogen receptor positive tumors have a significantly longer disease-free interval (McGuire et al., 1975; Wates et al., 1976; Knight et al., 1977; Maynard et al., 1978; Hihnel et al., 1979). In addition, a survival advantage has been reported for those patients with ER+ tumors (Wats et al., 1976).

The role of hormone receptors as prognostic and therapeutic tools has widespread acceptance in the management of breast cancer.

HER2/neu

HER2/neu protein over expression was first reported in In situ breast cancer by Van et al. in 1988. her 2/neu is located on chromosome 17 and also known as c-erbB-2 (HER-2) protooncogene and encodes a 185 kDa transmembrane phosphoglycoprotein with tyrosine kinase activity and is a member of the human epidermal growth factor receptor gene family. HER-2/neu protein, also called p185HER-2/neu, is derived from human epidermal growth factor receptor and it shows substantial homology with the epidermal growth factor receptor, EGFR (Slamon et al., 1988; De Potter et al., 1994).

HER-2/neu protein is a component of a four-member family of closely related growth factor receptors including EGFR or HER-1 (erb-B1); HER-2/neu (erb-B2); HER-3 (erb-B3) and HER-4 (erb-B4). Kurebayashi et al., 2001)

When HER-2/neu protein is over-expressed, tyrosine kinase is constitutively activated, resulting in mitogenic transduction and poor prognosis (Diaz et al., 2001; Gown et al., 2004; Bilous et al., 2003). Overexpression of this receptor have found in approximately 20%–30% of patients (Carney et al., 2003) and over-expressed in 10–20% of primary breast cancers (Jacobs et al., 1999; Diaz et al., 2001; Rhodes et al., 2004; Fitzgibbon et al., 2006; Wolff et al., 2007; Gown et al., 2008).

HER-2 gene amplification is found in 10–34% of invasive breast carcinomas and is regarded as an important prognostic marker indicating poor patient survival (Ross et al., 2003). Her-2/neu (c-erbB-2) gene amplification and this protein (HER-2) overexpressed in 15% to 25% of invasive breast carcinomas and is associated with a worse clinical outcome (Slamon et al., 1987; Ravdin et al., 1995; Ross et al., 1998; Kaptain et al., 2001; Bundred et al., 2001; Schnitt et al., 2001; Piccart et al., 2002; Chang et al., 2004; Yarden et al., 2004; Perez et al., 2004).

HER-2/neu protein overexpression, have been determined by immunohistochemistry and correlated with lymph node status, tumor grade (Berger et al., 1988). HER-2 expression generally is inversely correlated with ER and PR expression (Pous et al., 2001, Taucher et al., 2003). Slamon et al., (1987) reported a worse prognosis among Her2-positive breast cancers compared with Her2-negative tumors. (Slamon et al., 1987).

Recent reports suggested that HER-2 overexpression is significantly more likely in infiltrating ductal carcinomas than in infiltrating lobular carcinomas (Boussen et al., 2008). In contrast other studies showed that HER2/neu status was similar in both the in situ and invasive components of the single tumor confirming previous results (Allred et al., 1992; Barnes et al., 1992). This
growth factor receptor HER2/neu generally is not overexpressed in normal or benign breast lesions (Stark et al., 2000). According to alternative hypothesis HER2-negative ductal carcinomas do not derive from DCIS, but develop from ADH via an alternative pathway (Menard et al., 2001). Her2/neu expression is significantly lower in IDC/DCIS compared to IDC. It was suggested that IDC/DCIS may be a precursor for the development of a more aggressive and malignant IDC.

Human epidermal receptor protein-2 (c-erbB-2; HER2/neu) is known to be a prognostic as well as predictive marker in both node-negative and node-positive patients (Jacobs et al., 1999; Rhodes et al., 2004; Fitzgibbons et al., 2006; Gown et al., 2008). The overexpression of Her-2/neu protein and amplification of the Her-2/neu gene is also associated with poor prognostic tumor characteristics such as high histological grade, high proliferative index, negative or lower estrogen receptor (ER) expression, lymphoid infiltration, p53 mutation, absence of bcl-2, and absence of lobular histology (Pous et al., 2000; Ariga et al., 2005; Prati et al., 2005; Huang et al., 2005). Thus HER2 status might be incorporated into a clinical decision, along with other prognostic factors, regarding whether to give any adjuvant systemic therapy. More recent studies have identified HER2 as prognostic gene marker compared to other tumor markers. It have distinct biologic nature and found in triple negative (TN) patients (Haffty et al., 2006; Fan et al., 2006; Rakha et al., 2007). Carey et al. 2006 found the shortest survival among Her2+/ER and basal-like subtypes compared with other markers and growth factor receptor subtypes in North Carolina and African American women. Lower disease-free survival rate was found among hormone receptor (HR)-positive/Her2-/neu-positive patients compared with HR-positive/Her2-/neu-negative patients, all treated with tamoxifen (Gago et al., 2006). Many studies have shown that HER2/neu overexpression is an adverse prognostic factor (Paik et al., 1990; Tetu et al., 1994; Xing et al., 1996; Ross et al., 1998). Her-2/neu overexpressing tumors were shown to increase disease recurrence and metastasis and shorten survival.

Amplification of HER-2/neu gene is regarded as an established predictive and prognostic cancer biomarker for breast cancer, particularly for the management of advanced breast cancer. It is commonly used in current clinical practice to gauge breast cancer prognosis and identify appropriate treatment options (Duffy et al., 2005). HER2 status also appears to be predictive for either resistance or sensitivity to different types of chemotherapeutic agents (Carlomagno et al., 1996; Paik et al., 1998). Several studies on agents have shown the target HER2 are remarkably effective in both the metastatic and adjuvant settings. Testing for Her2 has become part of routine clinical practice. Trastuzumab (herceptin) in treatment form for metastatic disease approved by the FDA in November 1998 and for adjuvant treatment in 2006 (Slamon et al., 2001; Romond et al., 2005).

**BREAST CANCER SUBTYPES**

Breast cancer is heterogeneous disease, with variable morphological and clinical features. In recent years, breast cancer has been classified into five subgroups according to gene expression profiles: luminal A, luminal B, normal breast like, human epidermal growth factor receptor-2 (HER2) overexpressing and basal-like (Perou et al., 2000; Sorlie et al., 2001).

Breast cancer subtypes consist of two estrogen receptor (ER) positive types (Luminal A and Luminal B) and three ER-negative types (human epidermal growth factor receptor-2[HER2] expressing, basal like and normal breast-like) (Perou et al., 2000; Sorlie et al., 2001).

Sub classification of breast cancers patients have been proposed using IHC staining technique. Tumors were classified to the following subtypes: luminal A (ER+ and/or PR+, HER2), luminal B (ER+ and/or PR+, HER2+), HER2+ subtype (HER2+, ER/PR+), and basal-like (ER/PR+, HER2, cytokeratin 5/6+). Tumors that were negative for all 5 markers were considered unclassified (Sorlie et al., 2001; Sorlie et al., 2003; Nielsen et al., 2004).

Molecular profiling has provided biological evidence for heterogeneity of breast cancer through the identification of intrinsic subtypes. Analysis of gene expression data suggests that breast cancers can be divided into molecular subtypes which have distinct clinical features, with markedly differing prognosis and clinical outcomes (Perou et al., 2000; Sorlie et al., 2001; Sorlie et al., 2003; Sotiriou et al., 2003; Nielsen et al., 2004).

Immunohistochemistry (IHC) categorized breast cancer types into luminal, Her2, and basal like features using different markers (Makretsov et al., 2004). Recently published studies have used five IHC surrogate markers (ER, PR, HER2, CK5/6, and EGFR) for molecular class distinction (Carey et al., 2006; Tamimi et al., 2008; Tamimi et al., 2009).

Many subsequent studies have shown that breast carcinomas can also be divided into 5 similar subgroups using immunohistochemical (IHC) analysis with a limited panel of antibody markers (including ER, PR, HER2, CK5/6 and EGFR). (Nielsen et al., 2004; Livasy et al., 2006).

Luminal A tumors, characterized by positive ER and negative Her2 and it show the most favorable clinical features among the five subtypes (Nielson et al., 2004; Carey et al., 2006). Studies have shown that prognosis for the luminal subtype is mostly favorable, whereas for HER2 and BBC subtypes are poor (Brenton et al., 2005; Carey et al., 2006).

Perou et al., (2000) suggested a categorization of invasive breast cancers based on genetic profiles into estrogen receptor (ER) positive (luminal A and B) and ER-negative (non luminal) subtypes.ER-negative types further subdivided into Her2-positive and basal-like subtypes. Luminal A tumors differ from luminal B tumors by a higher expression of ER-related genes and lower expression of proliferation-associated genes.

The HER2+ tumors fall into at least 2 distinct groups: HER2+/ER- and HER2+/ER+ (Sorlie et al., 2001; Sorlie et al., 2003). Basal-like and Her2+ groups have been associated with poor clinical outcomes (Sorlie et al., 2001; 2003; Sotiriou et al., 2003; Nielsen et al., 2004).

Investigators have required TN status or ER-PR- negative and HER2-negative status for defining a BLBC in addition
to the expression of basal markers (Matos et al., 2005; Potemski et al., 2005; Kim et al., 2006; Carey et al., 2006; Pinilla et al., 2007) whereas other investigators did not consider hormone receptor and HER2 status (Van de Rijn et al., 2002; Laakso et al., 2005; Rakha et al., 2006; Laakso et al., 2006; Banerjee et al., 2006; Rakha et al., 2006; Fulford et al., 2006; Jumppanen et al., 2007; Fulford et al., 2007; Turner et al., 2007). These diverse breast cancers have been classified as basal-like breast cancers (BLBCs) with different prognostic significance. Several studies have demonstrated the expression of basal cytokeratins as a poor prognostic factor (Van de Rijn et al., 2002; Rakha et al., 2006; Banerjee et al., 2006). The basal cell like tumors has been associated with aggressive clinical, pathologic features and a poor prognosis (Carey et al., 2006). Most basal-like breast cancers (BLBCs) are estrogen receptor (ER)-negative, progesterone receptor (PR)-negative and HER2-negative (triple negative) at both transcriptional and translational levels (Nielsen et al., 2004; Livasy et al., 2006). Not only BLBCs but also normal breast-like cancers represent a triple negative (TN) phenotype (Rakha et al., 2007). Triple negative breast cancer (TNBC) can be further classified into two groups, BLBC and non-BLBC.

Many studies have been confirmed to identify the major molecular subgroups of invasive breast carcinoma (Nielsen et al., 2004; Rehimi et al., 2005; Jacquemier et al., 2005). TNBCs account for 11-32% of all invasive breast carcinomas (Carey et al., 2007; Dent et al., 2007; Bauer et al., 2007; Tischkowitz et al., 2007; Rakha et al., 2007; Sasa et al., 2008). Few studies have been reported on prognostic markers or therapeutic strategies for TNBCs (Tischkowitz et al., 2007; Rakha et al., 2007; Sasa et al., 2008). Many studies suggested the existence of more molecular classes (Pusztai et al., 2003; Sorel et al., 2003; Sotiriou et al., 2003) luminal A (LUMA), luminal B (LUMB), human epidermal growth factor receptor-2 (HER2) overexpressing, basal-like, and normal-like (Sorel et al., 2003). IHC used as a surrogate marker for gene expression profiling for further advancement. Breast cancer has recently been classified into subtypes according to gene expression profiles determined using cDNA microarrays (Perou et al., 2000; Sorel et al., 2003; Sotiriou et al., 2003). Gene expression profiling using DNA microarray analysis has led to the identification of 4 subtypes of breast tumors (luminal A, luminal B, basal cell–like, and Her-2/neu) characterized by distinct clinical and pathologic features and response to chemotherapy (Perou et al., 2000; Sorel et al., 2001; Sorel et al., 2003; Rouzier et al., 2005). Molecular diversification introduced a hybrid category comprising HER2+ luminal tumors. Diversities in the classification systems continue to emerge, but current studies still lack the potential to formulate a simple, practical, and easily applicable classification system for gene expression analysis that can completely and concisely encompass all the divergent facets of breast cancer (Bhargava et al., 2009).

Studies on whole-genome analysis using expression microarray have revolutionized our understanding of breast carcinomas which has led to the discovery of 5 distinct subtypes of breast carcinomas (Luminal A, Luminal B, HER2 over-expression, Basal-like, Normal-like) each with unique recognizable phenotypes and clinical outcomes (Perou et al., 2000; Sorel et al., 2001; Voer et al., 2002; Van de Vijver et al., 2002; Sorel et al., 2003). Gene expression microarrays has been shown intrinsic classification ER/PR+, Her2+ with Luminal B; ER/PR+, Her2+ with Luminal A; ER/PR-, Her2+ (ER-/Her2+) and ER/PR-, Her2- with triple negative/basal like tumors. (Carey et al., 2006; Carey et al., 2007). These subgroups have distinguishing features closely associated with subtypes defined by gene expression profiling including distinct clinical outcomes, different responses to adjuvant therapy and different patterns of metastatic recurrence (Carey et al., 2006; Hicks et al., 2006; Spitale et al., 2009; Cheang et al., 2009).

**Triple Negative breast cancer (Nonaggressive cancer)**

Women with nonaggressive type of cancer are ER+ breast cancer typically receive endocrine therapy (tamoxifen or aromatase inhibitors) and women with Her2+ breast cancer may receive anti-Her2 (trastuzumab, Herceptin) and lapatinib.

Breast cancer with aggressive type includes the triple-negative (Tischkowitz et al., 2007; Dent et al., 2007; Colleoni et al., 2010) and basal-like breast cancer (Rehimi et al., 2004) which tend to be larger than other subtypes of breast cancer and usually of high-grade, invasive ductal carcinomas. Studies have shown basal-like breast cancers are more likely than other types of breast cancer to be node-negative (Cheang et al., 2008).

Triple-negative breast cancer has become a commonly used descriptor for malignancies that are estrogen receptor, progesterone receptor and HER2 negative. Histological, triple-negative tumors were mainly of high histological grade (grade 3), largely ductal (Carey et al., 2006) but several unusual histologies also overrepresented, including metaplastic (Livasy et al., 2006; Filho et al., 2006; Beatty et al., 2006) high mitotic index, and were found more frequently in premenopausal women (Umemura et al., 2005). Aggressive subtype of breast cancer marked by higher rates of visceral and central nervous system metastases and poorer disease-specific survival than hormone receptor–positive subtypes (Osborne et al., 2003; Carey et al., 2006; Pinilla et al., 2006; Dent et al., 2009).

Women with triple negative breast cancer were slightly younger. Body size may also be associated with a woman’s risk for developing postmenopausal triple-negative breast cancer, either through an elevated risk in women with a high weight or through a reduced risk in women with a low weight.

Treatment strategies for patients with triple-negative breast cancer are many chemotherapy agents such as the anthracyclines, taxanes, ixabepilone, and platinum agents as well as selected biologic agents and possibly anti-EGFR drugs. Platinum therapy in particular requires close scrutiny because preclinical evidence suggests that it may be especially useful in triple negative breast cancer. Combination of iniparib with gemcitabine–carboplatin provides significant clinical benefit with a favorable safety profile in patients with metastatic triple-negative breast cancer. Reported prospective of clinical trials of bevacizumab is complicated and there is no clear signal...
about any special anti angiogenic properties in the triple negative BC.

CONCLUSION

Although significant progress has been made in both the understanding and treatment of cancer during the last thirty years, it remains the second leading cause of death in the US. The cancer community has set a goal to eliminate cancer-related suffering and death by 2015. To achieve this goal, not only better therapies are required but also improved methods to assess an individual’s risk of developing cancer to detect cancers at early stages when they can be treated more effectively to distinguish between aggressive and nonaggressive cancers and to monitor recurrence and response to therapy. Recent advances in high-throughput technologies in genomics, proteomics and metabolomics have facilitated biomarker discovery. As more potential biomarkers are discovered, further studies are needed to validate these markers. The ultimate use of these biomarkers is in clinical applications for cancer detection and treatment. Many steroid receptors have been used in breast cancer for predicting outcome and response to therapy for many years. Presently, we lack the targeted therapies for triple negative breast cancer and this continues to direct the focus of ongoing research.

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