AJMHR
Asian Journal of Medical and Health Research
Journal home page: www.ajmhr.com

Prognostic and diagnostic role of putative biomarkers in breast carcinoma: An overview

S. Mirunalini *[1], K. Deepalakshmi[1]
1.Department of Biochemistry & Biotechnology, Annamalai University, Tamil Nadu, India.

ABSTRACT

This review illustrates the relationship between biomarker and breast cancer and their molecular aspects which referring to both drugs and therapies. Even though, various prediction models have been constructed for breast cancer therapy, established clinopathological factors are not sufficient for clinical decision making particular regarding adjuvant chemotherapy. Some of the key decisions in the current management of breast cancer involve the need for prognostication; which is especially important in identifying patients whose prognosis is so favorable or patients whose prognosis is poor with conventional treatment as to warrant consideration of more aggressive investigational therapies. Several major programs have been organized to facilitate the validation and assessment of cancer molecular marker alongside the established “standards of care” for cancer diagnosis and treatment. Despite their successes, it is now commonly accepted that molecular marker have independent predictive power for disease predisposition, early detection, cancer staging, therapy selection, identifying weather or not a cancer is metastatic, therapy monitoring, assessing prognosis and advanced in the adjuvant setting. In this review, markers have been discussed essentially by highlighting the molecular aspects and referring to both drug and therapies, only as overviews.

Keywords: Breast cancer; Tumor markers; Ki-67; Tumor necrosis factor; Caspases.

*Corresponding Author Email: mirunasankar@gmail.com
Received 16 March 2016, Accepted 28 April 2016

Please cite this article as: Mirunalini S, Prognostic and diagnostic role of putative biomarkers in breast carcinoma: An overview. Asian Journal of Medical and Health Research 2016.

INTRODUCTION

Breast cancer continues to be a leading cause of cancer-related death and the most common cancer among women with prevalence increasing with age (Hanahan and Weinburg 2011)[1]. The development of breast cancer and metastasis is a multistep process that often involves alterations and defects in major cellular pathways including DNA damage response, proliferation, senescence, angiogenesis and usually accompanied by an extended chromosomal instability (Kryston et al. 2011)[2] . Even though, various prediction models have been constructed for breast cancer prognosis using clinical risk factors and environmental exposures. Despite their successes, it is now commonly accepted that genomic markers have independent predictive power (Cheang et al. 2008)[3]. In present days researchers currently focused on understanding the clinical significance of known markers finding relationships between them and discovering ones (Giancotti 2006)[4]. An avenue that is currently being actively pursued in clinic as well as translational research is looking at various molecular and biological marker often called "biomarker".

A biological marker is a characteristic that is objectively measured and evaluated in biological samples as an indicator of conditions like normal biological processes, pathogenic states or pharmacologic responses to a therapy by a various techniques. However, molecular marker can be further classified as diagnostic, prognostic and predictive markers (Sawyers 2008)[5]. According to these diagnostic biomarkers can help in disease diagnosis. Prognostic markers that are utilized extensively by clinicians can be correlated with an endpoint regardless of therapy. On the other hand, predictive biological indicators predict outcome to specific therapy (Nowsheen et al. 2012)[6]. In this context, we presently summarized the most important currently available biomarkers in breast cancer that provide prognostic or predictive information.

Etiology and major risk factors

Breast cancer is a neoplastic process with a multifactorial etiology. Several factors, which act simultaneously and /or sequencelly, regulate the different steps of mammary carcinogenesis. These factors can be classified in the groups endocrinological, genetic and environmental. Among them, environmental factor and in particular nutrition represent an important group because of their transcendence in the population. Lipid intake has received the most attention as a possible risk factor of all the aspects of dietary composition that may be related to breast cancer. In general age is the strongest risk factor for breast cancer. Unlike many cancers that increase beginning at the end of the fifth decade of life, breast cancer begins to rise in the third decade of life, breast cancer begins to rise in the third decade of life, most likely due to the effects of ovarian hormones on breast tissues (Hulka and Moorman 2001)[7] . Morethan 2/3 of all new cases occur after the age of 55 and women older than 65 have a relative risk greater than 4.0 when compared with those younger than 65. Other factors that can increase the risk of breast cancer include an increase in lifetime exposure to endogenous or exogenous estrogen has been implicated as the most important risk factor for breast cancer. In addition exposure to environmental pollutants, including polycyclic aromatic hydrocarbons, during critical phases of early development is known to play a vital role in breast cancer susceptibility during the latter period of life.

Diagnosis

Clinical characteristics as age, menstrual status, tumor size, lymph node status and morphological characteristics of the tumor (histological type, Grade, lymphatic/vascular invasion) are an important prognostic factors. Traditional triple test for breast cancer diagnosis includes physical examination, mammography and aspiration cytology (Mitika 2003)[8]. Some of the diagnostic tools have been listed below:

Mammogram: The first diagnostic tool to identify breast cancer is mammogram is an X-ray of the breast that can show the presence of abnormal growth lumps in the breast area (Qaseem et al. 2007)[9].

Biopsy: The removal of cells or tissues so they can be viewed under a microscope by a pathologist to check for signs of cancer. Four types of biopsies are as follows:

- **(i) Excisional biopsy:** The removal of an entire lump of tissue.
- **(ii) Incisional biopsy:** The removal of part of a lump or a sample of tissue.
- **(iii) Core biopsy:** The removal of tissue using a wide needle.
- **(iv) Fine-needle aspiration (FNA) biopsy**: The removal of tissue or fluid, using a thin needle.

MRI (magnetic resonance imaging): A procedure that uses a magnet, radio waves, and a computer to make a series of detailed pictures of areas inside the body. This procedure is also called nuclear magnetic resonance imaging (NMRI).

Lack of sensitivity for early malignancy and lack of specificity, combined with the low prevalence of most cancers in the general population; preclude the use of most existing tumor markers for screening asymptomatic subjects for early malignancy (Roulston 1990)[10]. Despite these limitations, a number of tumor marker markers have either undergone or are currently undergoing evaluation as potential cancer screening test. Tumor marker can be used as an adjunctive tool to narrow down a differential diagnosis of many but not all cancer type. Such marker may be used in the primary prevention of cancer, but also in screening, secondary prevention, diagnosis, prognosis, recurrence and monitoring of disease status.

Molecular biomarkers

Molecular biomarkers include altered or mutant genes, RNAs, proteins, lipids, carbohydrates and small metabolite molecules, and their altered expressions that are correlated with a biological behaviour or a clinical outcome. The role of biomarkers in cancer detection and progression is a major effort at various laboratories aimed at the development of novel and simple approaches for early detection of human cancer. Molecular profiling studies, the major contributors of cancer biomarker discoveries, are based on an association or correlation between a molecular signature and cancer behavior. One of the pioneering molecular profiling studies showed that gene expression patterns could classify tumors, yielding new insights into tumor pathology such as stage, grade, clinical course, and response to treatment (Grimm et al. 2013)[11]. (Table 1) contain list of test acronyms used in tumor biomarker detection.

Table 1: List of test acronyms used in tumor marker detection

Test acronyms used in tumor marker detection	
EIA	Enzyme immunoassay
FISH	Fluorescent in-situ hybridization
ICC	Immunocytochemistry
ICMA	Immunochemiluminometric assay
IHC	Immunochemistry
IRMA	Immunoradiometric assay
MEIA	Microparticle enzyme immunoassay
PCR	Polymerase chain reaction
RIA	Radioimmunoassay
RT-PCR	Reverse transcriptase polymerase chain reaction

Characteristic features of tumor molecular markers

As a consequence of that, some criteria were chosen for the validation and proper selection of the most appropriate marker in a particular malignancy, and these are:

1. **Markers' sensitivity:** Sensitivity expresses the mean probability of determining an elevated tumor marker level (over the "cut-off value") in a tumor-bearing patient.
2. **Specificity:** Specificity expresses the mean probability that a normal tumor marker value derives from a tumor-free individual.
3. **Predictive values:** The predictive value shows the applicability of a tumor marker in a mixed group of patients.

Many theoretical applications exist for tumor markers in clinical oncology. Clinically important utilization of markers includes

(i) Early detection of the tumor

(ii) Differentiating benign from malignant conditions

(iii) Evaluating the extent of the disease

(iv) Monitoring the response of the tumor to therapy, and

(v) Predicting or detecting the recurrence of the tumor.

Since no ideal tumor markers with adequate sensitivity and specificity currently exist, they are only exceptionally used in screening (prostate specific antigen - PSA). Nevertheless, tumor markers can play a crucial role in the detection of an early disease relapse and assessment of response to therapy in selected groups of patients. In monitoring the patients for disease recurrence, tumor marker levels should be determined only when meaningful treatment is possible (Novakonic 2004)[12].

Classification of tumor molecular markers

According to their application, tumor molecular markers can be roughly divided as markers in clinical oncology and markers in pathology. Current tumor markers in clinical oncology include

(i) Oncofetal antigens

(ii) Placental proteins

(iii) Hormones

(iv) Enzymes

(v) Tumor-associated antigens

(vi) Special serum proteins

(vii) Catecholamine metabolites, and

(viii) Miscellaneous markers

The Potential uses of tumor markers are screening in general population, differential diagnosis in symptomatic patient, clinical staging of cancer, estimating tumor volume, prognostic indicator for diseases progression, evaluating the success of treatment, detecting the recurrence of cancer, monitoring responses to therapy, radio immunolocalization of tumor masses, Determining direction of immunotherapy.

***In vivo* factors that affect Tumor markers**

(i) Elevated values may be observed in renal failure and cholestasis, due to impaired excretion of the markers.

(ii) In rheumatic diseases, elevated levels of CA-19-9 are observed.

(iii) Drug interactions: antiandrogens inhibit PSA production.

(iv) Rectal examination or transuretheral manipulation results in elevation of serum level of PAP and PSA.

(v) Cigarette smoking can result in elevation of CEA levels up to 10ng/ml (Novakonic 2004)[12].

As to the literature, an ideal tumor marker should fulfil certain criteria - when using it as a test for detection of cancer disease. Positive results should occur in the early stages of the disease.

1. Positive results should occur only in the patients with a specific type of malignancy.
2. Positive results should occur in all patients with the same malignancy.
3. The measured values should correlate with the stage of the disease.
4. The measured values should correlate to the response to treatment
5. The marker should be easy to measure. Most tumor markers available today meet several, but not all criteria.

Molecular markers in mammary carcinogenesis

Molecular biomarkers include altered or mutant genes, RNAs, proteins, lipids, carbohydrates and small metabolite molecules, and their altered expressions that are correlated with a biological behaviour or a clinical outcome. Molecular profiling studies, the major contributors of cancer biomarker discoveries, are based on an association or correlation between a molecular signature and cancer behaviour. One of the pioneering molecular profiling studies showed that gene expression patterns could classify tumors. (Table 2) shows the classification of bio markers.

Table 2: Biomarker for mammary cancer.

Sr.No	Types	Bio-markers
1	Proliferation Marker	ER,PR,HER-2,Ki-67,PCNA
2	Inflammatory Marker	TNF-α, COX-2,NFκB
3	Apoptotic Marker	Bcl-2,Bax,Fas-FasL, Caspase -3,-8,-9, P53, cyclin D1
4	Angiogenesis Marker	VEGF, BRCA1 and BRCA2
5	Enzymes	Aromatase Inhibitor,MMP13

Proliferation Markers

The tumour proliferation rate is an important prognostic factor in breast cancer. It is essential to determine their ability to predict prognosis or response to therapy or both (Table 3). Several methods have been developed to estimate the proliferative rate of tumour cells. The S-phase fraction, as measured by flow cytometry, is a validated method for measuring tumour proliferation (Clark et al.1989)[13]. However, flow cytometry is not commonly used because of the amount of tissue consumed for the assay. Alternative methods for measuring tumour proliferation have been developed, including immunohistochemistry (IHC) to detect cell cycle-related antigens, that are better suited for the evaluation of small archival tissue samples.

Table 3: Prognostic factors in breast cancer

Well established Prognostic factor	Investigational Prognostic factor
Ki-67	pS2
Estrogen receptor	Mitosin
Progestron receptor	Epidermal growth factor receptor
HER-2	Insulin-like growth factor
	Apoptosis-related proteins
	Cell cycle molecules
	Plasminogen activators and inhibitors
	Angiogenesis-related proteins

Hormone Receptors

Hormone receptors are proteins expressed both in the epithelium and in breast stroma which bind to circulating hormones, mediating their cellular effects (Haslam 1989)[14]. According to the college of American pathologists both the estrogen receptor (ER) and progestrone receptor (PR) constitute a first category of prognostic factor in breast cancer. Early evidence suggesting a hormonal role in breast cancer development began with an early observation that bilateral oophorectomy significantly reduce breast cancer risk, and that risk reduction is greater if the ovaries are removed earlier in life (Tricbopoulous et al.1972)[15].

Estrogen receptor

Estrogen is essential for growth and development of the mammary glands and has been associated with the promotion and growth of breast cancer (Speirs et al. 2002)[16]. Estrogen receptor (ER) of which two isoforms have been detected (α and β) are members of the steroid/ thyroid hormones superfamily of nuclear receptor (Girdler and Brotherick 2002)[17]. All steroid hormones originate from C27 cholesterol. The main source of cholesterol required for the synthesis of steroid hormones (steroidogenesis) is LDL-cholestrol (Carr et al. 1982)[18]. In ovarian steroidogenesis the movement of cholesterol into mitochondria is the initial step regulated by steroidogenic acute regulatory protein (*StAR*) encoded by STAR gene (Miller and Strauss 1999)[19]. Further the conversion of cholesterol to pregnenolone, catalysed by mitochondrial side-chain cleavage enzyme complex. Pregnenolone act as precursor for all steroid hormones it metabolized by several enzymes and converted to progesterone or androstendione. Androstendione in turn is further metabolized to other androgens or estrogens.

Estrogen receptors regulate the expression of genes involved in cell proliferation and/or differentiation. Estrogen mechanisms of breast cancer were illustrated in (Figure. 1). Binding of an estrogen (or an antiestrogen) cause a conformational change in both receptor types leading to their dimerization, strong association with DNA and recruitment of co-activators (or co-repressors) as well as other transcription factors. However, evidence suggesting a hormonal role in breast cancer development began with an early observation that bilateral oophorectomy significantly reduces breast-cancer risk, and that risk reduction is greater if the ovaries are removed earlier in life (Tricbopoulous et al. 1972)[15].

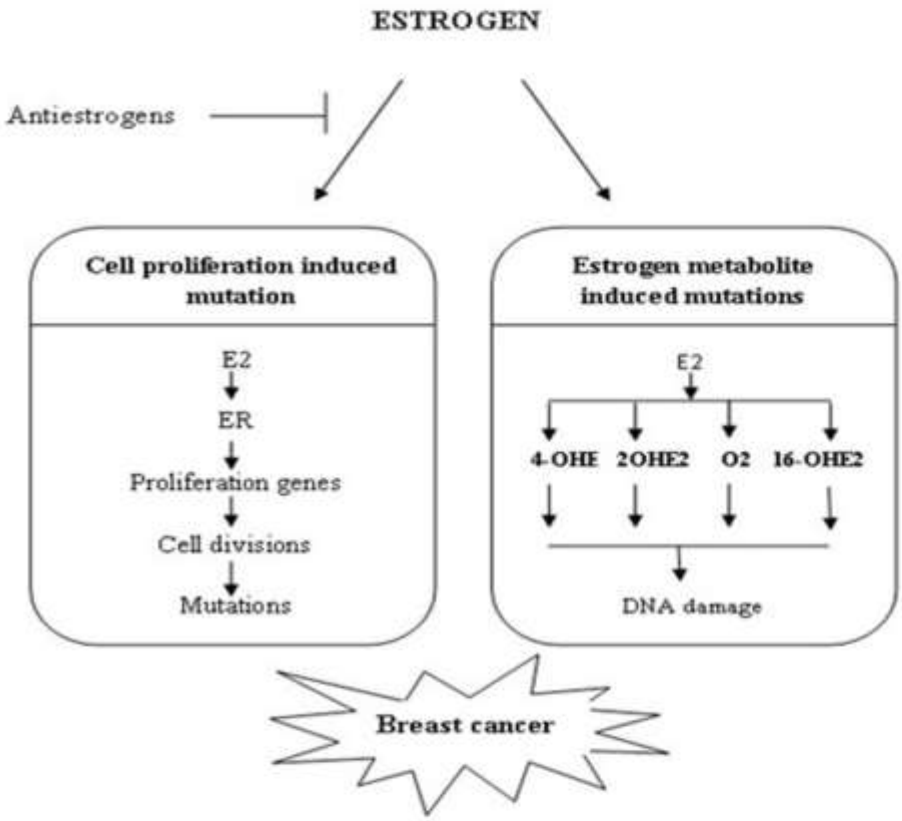

Figure 1: Estrogen mechanism of Breast cancer

Progestrone receptor

Progestrone (P4) is a key cycling ovarian steroid hormone that is highest in the luteal phase and has a major role to promote glandular differentiation of the endometrium. P4 is also sustained at high levels during pregnancy and is required for maintenance of pregnancy (Anderson and Clarke 2004)[20]. Based on immunohistochemistry progesterone receptor (PR) is exclusively expressed in the epithelial cell compartment of mammary gland ducts with no evidence of expression in myoepithelial cells or stroma (Ismail et al. 2003)[21]. PR is uniformly expresses in epithelial cells in juvenile mammary gland ducts but switches to a heterogeneous pattern during puberty and in the adult. PR is expressed in approximately 40% cells and PR+ve cells are largely non-proliferative and reside nearly proliferative PR negative cells suggesting a paracrine mechanism for P4 induced proliferation (Shyamala et al. 2002)[22].

The mechanism(s) underlying progesterone as a breast cancer risk factor is not well defined, but hypotheses have been developed. Whereas, the life-time cyclical proliferative effect of P4 on the breast cancer initiated by specific genetic changes. Indeed exposure of the human breast epithelium to ovarian sex steroids during the reproductive years is established to be a risk factor for breast cancer. As proliferative hormone, progesterone is a risk factor for human breast cancer and that it stimulates normal human breast epithelium through a paracrine mechanism in (Figure. 2) (Bernstein 2002)[23]. Alterations in the progesterone/PR signalling axis, including a switch from a paracrine to an autocrine regulation of proliferation contribute to progression. In more advanced stage breast cancer PR, either independent of P4 or in response to P4, suppress tumor invasion and metastasis through maintaining epithelial cell phenotype and impeding the epithelial- mesenchymal transition (EMT).

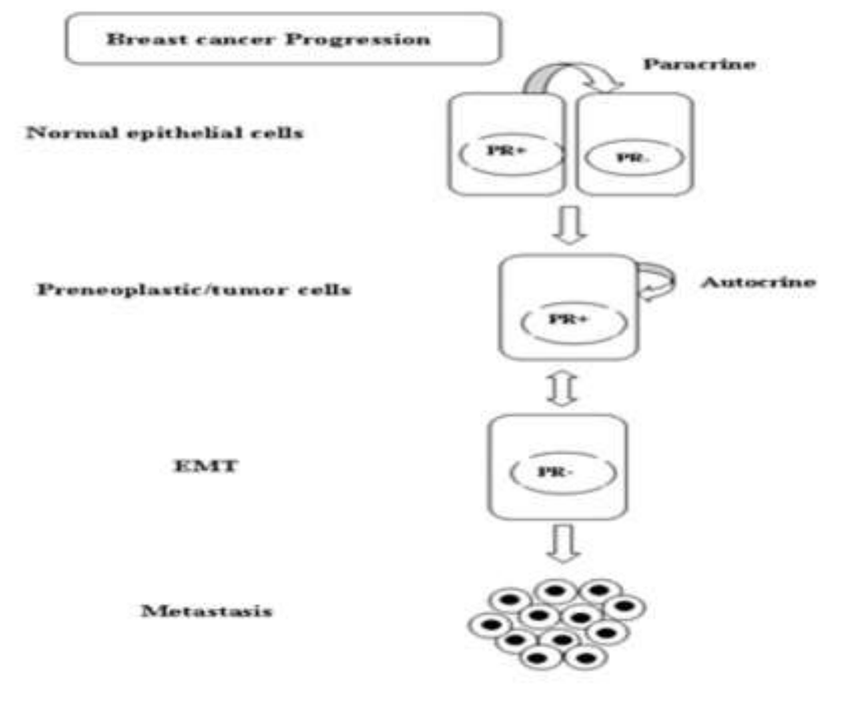

Figure 2: Progestron mechanism of cancer

HER-2

HER 2/*neu* (human epidermal growth receptor 2) also known as ErB2, is a protein marker for breast cancer, which is currently used to predict response to trastuzumab (Herceptin) based treatment and to prognosticate course of diseases. There are two possibilities to measure the molecular state of HER-2/*neu* in patients: Immunohistochemistry (IHC) staining of whole cell membrane receptor in tissue sections obtained from biopsy or decetion of the concentration of the extracellular domain (ECD) of HER-2/*neu* (HER-2/*neu*-ECD) which is shed in serum, via ELISA featuring the advantage of a biopsy–free analysis (Payney et al. 2000)[24].

HER-2 is a receptor regulates a wide range of cellular process, including proliferation, differentiation, motality, survival, angiogenesis, invasion and antiapoptotic functions (Harari and Yarden 2000)[25]. An endogenous ligand for the HER2 receptor has not been identified, but its activation is through to occur through heterodimerization when highly expressed. Herceptin is also known as Trastuzumab, is a humanized monoclonal directed against the extracellular domain of HER-2. From the previous studies have shown that herceptin inhibits the growth of both HER-2 positive breast cancer cells in culture and HER-2 positive tumor in animals (Sliwakowshi et al. 1999)[26]. Furthermore, administration of Herceptin with chemotherapy resulted in better response rates, longer time to diseases progression and longer survival that chemotherapy alone (Shaks 1999)[27]. Thus, HER-2 assay is currently mandatory in deciding wheather or not to treat breast cancer patients with herceptin.

Ki-67

The Ki-67 antigen was originally identified by Gerdes and colleagues in the early 1980s, by use of a mouse monoclonal antibody against at nuclear antigen from a Hodgkin's lymphoma-derived cell line. This non-histone protein was named after the researchers location, Ki for Kiel university, Germany, with the 67 lable referring to the clone number on the 96- well plate (Gerdes et al. 1983)[28].

Ki-67 is a nuclear antigen found in cells during the proliferative phase of the cell cycle (G1 phase, S phase, G2 phase and M phase), but not in cells during the resting phase (G0 phase). The protein can be identified by the monoclonal antibody MIB-1. Various studies have described the prognostic significance of the proliferation marker Ki-67 in invasive and early breast cancers. Whereas, patients whose tumors overexpress Ki-67 in more than 50 % of the cells are at high risk of developing recurrent disease (Urruticoechea et al. 2005)[29]. In addition, Ki-67 can serve as tool to identify patients who will benefit from a specific chemotherapy or endocrine treatment. Therefore Ki-67 might have a valuable role in predicting benefit from specific treatment in subtypes of breast cancer.

Proliferating cell nuclear antigen (PCNA)

Proliferation cell nuclear antigen (PCNA) has been called the "ringmaster of the genome", because this 29-kDa protein has been shown to actively participate in a number of the molecular pathways responsible for the life and death of the mammalian calls. PCNA by cells during the S and G2 phase of the cell cycle makes the protein a good cell proliferation marker. Cell proliferation is a biological process of vital importance to all living organism both in embryonic and in post embryonic existence. Down regulation of cell proliferation process is an important biological process lost in cancer. It is a member of cyclin family, an auxillary protein to DNA polymerase and is involved in replication and repair process of DNA. Increase in PCNA expression has been reported when tissue progresses from normal epithelium to hyperplasic dysplasia and cancer (De Biasio and Blanco 2013)[30].

Inflammatory Markers

Many cancers arise from sites of infection, chronic irritations and inflammation. It is now becoming clear that the tumor microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. In addition, tumor cell have co-opted some of the signalling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis. These insights are fostering new anti-inflammatory therapeutic approaches to cancer development (Lisa et al. 2002)[31].

Tumor necrosis factor alfa [TNF-α]

TNF-α is a 17 kDa protein consisting of 157 amino acids that is a homotrimer in solution (Muller et al. 1987)[32]. Although TNF-α is a pleiotropic cytokine that can regulate a systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reactions. TNF-α is mainly synthesized by activated macrophages, NK cells, T cells, B cells and natural killer cells (Carswell et al. 1975)[33]. TNF-α was originally identified as an endotoxin induced, macrophages- derived serum protein that has ability to induced necrosis of tumor (Goldberg et al 2010)[34] .TNF-α receptor activation requires formation of multiprotein signalling complex leading to activation of transcriptional or an apoptotic pathways (Wu et al. 1993)[35]. Although TNF-α was originally characterized to cause hemorrhagic tumor necrosis at high concentration in many types of cancer, low concentrations of TNF-α seem to increase tumor growth and progression (Balkwell 2002)[36].

TNF-α expression inflammatory breast carcinoma was found to be related to higher tumor grade and lymph node involvement. The tumor- promoting function of TNF-α may be mediated by its ability to induce proangiogenic functions, to promote the expression of matrix metalloproteinase (MMP) and endothelial adhesion molecules and to cause DNA damage via reactive oxygen (Storci et al. 2010)[37] . In addition TNF-α act as a mediator for IL-6 and IL-8 production. It also induces NF-κB signalling pathway activation in stem like phenotype (Turini and Dubois 2002)[38].

COX-2

Prostaglandin endoperoxide synthase, commonly called cyclooxygenase (COX), is ~ 68 kDa protein is the key enzyme required for the conversion of archidonic acid to prostaglandins. COX-1 and COX-2 are two known COX isoforms, COX-1 is higher inducible (eg., in gastric mucosa) whereas COX-2 is highly inducible (eg., at sites of inflammation and cancer) (Figure. 3) (Davies et al. 2002)[39].

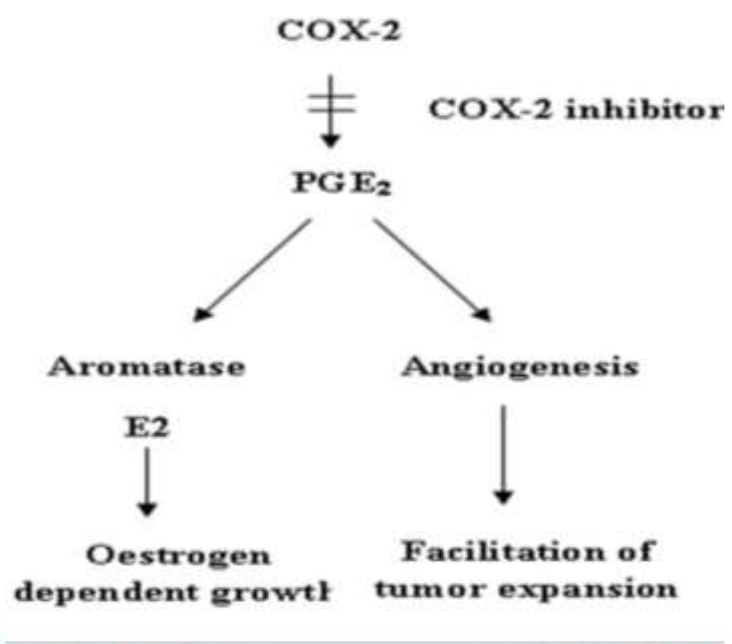

Figure 3: COX-2 inhibitors as chemopreventives of breast cancer (Davies et al. 2002)[39].

NFκB

The nuclear factor κB (NF-κB) was discovered as a protein bound to the kappa immunoglobulin gene enhancer in the nuclei of B cells (Sen and Baltimore 1986)[40]. The proteins are a family of transcription factors that regulate expression of genes involved in immune and inflammatory responses, cell growth, differentiation and apoptosis (Junghan and Arnold 2004)[41] .

NF-κB is required for normal lobuloalveolar development of mammary gland. whereas, deregulation of normal NF-κB, activity such as expression of an abnormal form of the normal gene, has been shown to be involved in development of leukemia, lymphomas and solid tumors (Rayet and Gelinas 1999)[42]. The transcriptional activity of NF-κB is regulated by two pathways, termed the canonical and non-canonical pathways both pathways have now been implicated in carcinogenesis (Annunziata et al. 2007)[43]. In general inflammation and NF-κB in particular have a double-edged role in cancer. Whereas, NF-κB activation usually results in the up-regulation of anti-apoptotic genes there by providing cell survival mechanism to withstand the physiological stress that triggered the inflammatory response (Perkins 1997)[44]. Furthermore, NF-κB induced cytokines that regulated the immune response (such as TNF-α, IL-1,IL-6 and IL-8) as well as adhesion molecules, which leads to the recruitment of leukocytes to sites of inflammation. Moreover, NF-κB signaling was shown to contribute to cancer progression by controlling epithelial to mesenchymal transition and metastasis (Huber et al. 2004)[45]. NF-κB also controls the expression of several genes that regulate cell cycle (cyclin D1), differentiation (P21cip/Waf 1), cell survival (Bcl-2, Bcl-XL, cIAP), growth factor (VEGF), cell adhesion and angiogenesis.

Apoptotic Marker

Apoptosis (or) programmed cell death is a genetically controlled cell death process, which is characterized by chromatin condensation, DNA fragmentation to nucleosome-sized pieces, membrane blebbing, cell shrinkage and compartmentalization of the dead cells into membrane enclosed vesicles or apoptotic bodies (Silva et al. 2014)[46]. It is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. In general, apoptosis can be induced by two major pathways: extrinsic or death receptor mediated pathways and intrinsic or mitochondrial mediated pathways.

Bcl-2 associated x-protein (Bax)

Bcl-2 associated x-protein (Bax) is one of the primary targets of P^{53} and controls cell death through its participation in disruption of mitochondria with the subsequent release of cytochrome c in cytosols (Marzo et al. 1999)[47]. Bax is a 21-kDa protein that share homology with Bcl-2 in conserved region, including Bcl-2 homology domains BH1 and BH2. It may heterodimer zed with Bcl-2 or other proteins and homodimerize with nucleus in viable cells.

Bax is a cytosolic monomer, however during apoptosis, it can changes its conformation and inserts into the outer mitochondrial membrane thereby it may form oligodimers. Bax oligomers are believed to contribute to the permeabilization of the mitochondrial membranes, either by forming channels, by interacting with components of the permeability transition pore (PTP) or by altering fission and fusion processes. Moreover it may interact with the BH3 domain or heterodimerized with Bax, thereby preventing Bax oligomerization and leading to an inhibitor of the mitochondrial pro-apoptotic events. Since Bax and Bcl-2 levels may regulate cell death processes and both proteins are widely expressed in many tissues, the Bcl-2-to-Bax ration varies in different cells, systems and during development or in the presence of apoptotic signals (Liu et al. 2013)[48].

B-cell lymphoma gene-2 (Bcl-2)

Bcl-2 was initially discovered in human B-cell lymphoma 2. It is a 24-kDa protein and has been shown to be located in the mitochondrial membrane, smooth endoplasmic reticulum and nuclear membrane. Based on their functions the members of the Bcl-2 family can be divided into pro-apoptotic (such as Bad and Bax) and pro-survival (or) anti-apoptotic (such as Bcl-2 and Bcl-XL) proteins.

The Bcl-2 protein binds to pro-apoptotic protein Bax and form heterdimers and the molar ratio of Bax to Bcl-2 determines whether apoptosis is induced or inhibited in the target tissue (Oltvai et al 1993)[49]. When there is an excess of pro-apoptotic proteins, the cells are more sensitive to apoptosis and when there is an excess of anti-apoptosis proteins, the cells will tend to be less sensitive to apoptosis Bcl-2 is a key regulator of apoptosis and plays an essential role in cancer and chemo resistance. It also contributes to neoplastic cell expansion cell expansion by preventing normal cell turnover caused by physiological cell death mechanisms. High levels of Bcl-2 gene expression are found in wide variety of human cancers and correlate with relative resistance to current chemotherapeutic drugs and γ-irradiation (Cunha et al. 2013)[50].

Fas/FasL

The cell surface Fas receptor (Fas), also termed as Apo 1 or CD95, is a member of the tumor necrosis factor (TNF) family of receptor (TNF-R), a group of type I transmember proteins. Structurally, Fas is a transmembrane cell surface receptor containing three cystein- rich extracellular domains at the amino- terminus, which are responsible for ligand binding and an intracellular death domain (DD) of about 80 amino acid that is essential for transducing the apoptosis signal (Peter and Krammer 2003)[51].

In higher organisms have developed several mechanisms to ensure the rapid and selective elimination of unwanted cells, one of which involves the interaction of cell surface Fas with its cognate ligand, Fas L (Figure. 4) (Houston and Connell 2004)[52]. Binding of FasL to Fas cause a higher order aggregation of the receptor molecules and requirement of the adaptor molecules Fas- associated death domain (FADD) via DD-DD interactions. FADD also has another domain called death effector domain, in which in turn recruits pro-caspase-8 (FLICE) and/or pro-caspase -10 to the receptor. The resulting multimeric protein complex is called the death-inducing signaling complex (DISC), and forms within seconds of receptor engagement (Peter and Krammer 2003)[51]. At the DISC, caspase-8 is activated which leading to the rapid activation of caspase-3 and cell death are known as type I cells. In some cells however, DISC formation following Fas stimulation is strongly reduced known as type II cells, in these cells mitochondria play an essential role as signal amplifies (Barnchart et al. 2003)[53].

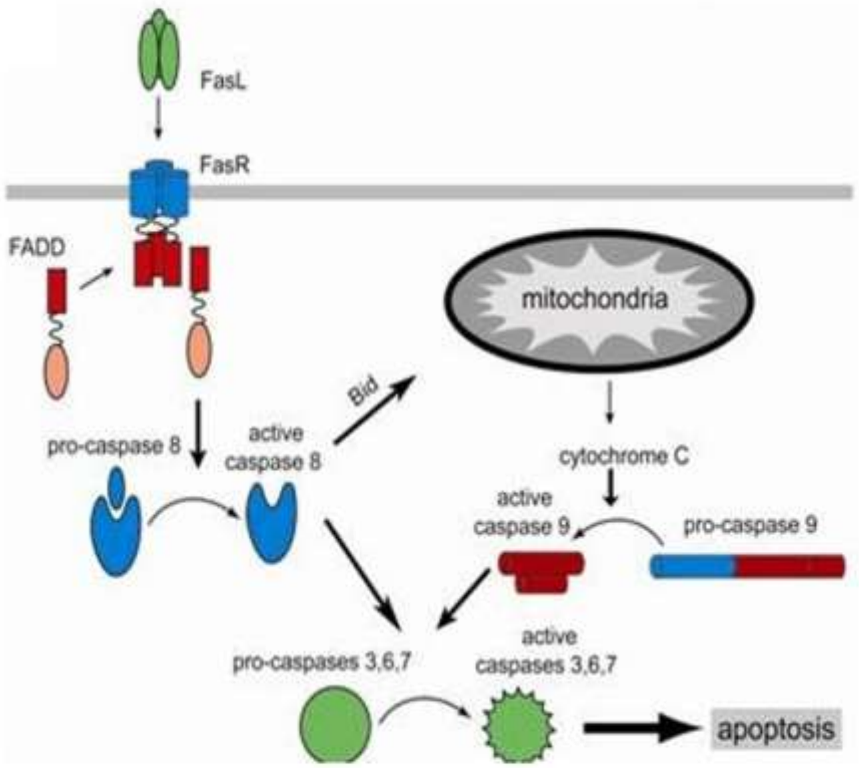

Figure 4: Apoptotic signaling via Fas receptor (Houston and Connell 2004)[52] .

Caspases and its inhibitors

The CASPASE (**C**ysteinyl **A**spartate **S**pecific **P**rote**ASEs**) are family of important signaling molecules with various tasks depending on various subtype and organ involved. Currently in human, the caspase family consists of 13 members. Caspases are synthesized as relatively inactive zymogens that can be activated by removal of the regulatory prodomain and assembled into the active heteromeric protease (Nicholson 1999)[54]. There are two types of apoptotic caspases: initiator (apical) caspases and effector (executioner) caspases.

Initiator caspases (e.g. CASP2, CASP8, CASP9, and CASP10) cleave inactive pro-forms of effector caspases, thereby activating them. Effector caspases (e.g. CASP3, CASP6, CASP7) in turn cleave a variety of intracellular protein substrates within the cell to trigger the apoptotic process. These protein targets include major structural elements of the cytoplasm and nucleus, components of the DNA repair machinery, and a number of protein kinases. All major apoptotic pathways result in the activation of caspases (Khan et al. 2006)[55].

Caspase 3

Caspases are synthesized as inactive proenzymes and become activated either by oligomerization in a large multimeric complex, which is the case for the initiator caspases-8 and caspase-9 or alternatively via proteolytic cleavage, which applies for effector caspase such as caspase-3 (Degterev et al., 2003)[56]. Caspase -3 is activated by the upstream caspase-8 and caspase-9 and science it serves as a convergence point for different signaling pathways.

Once activated, they cleave various substances in the cytoplasm or nucleus causing characteristic morphological features of apoptotic cell death.

Pathways to caspase-3 activation have been identified are either dependent on or independent of mitochondrial cytochrome C release and caspase-9 function. Caspase-3 is an effectors caspase that is activated through the mitochondrial pathway that involves caspase- 9 or a death receptor pathway that involves caspase 8. Caspase-3 is a vital molecule in the apoptosis cascade, and the relationship between caspase-3 expressions and prognosis has been reported in many types of malignancies. Moreover it is also required for some typical hallmarks of apoptosis, and is indispensable for apoptotic chromatin condensation and DNA fragmentation, Thus it may also function before or at the stage when commitment to loss of cell viability is made (Degterev et al., 2003)[56].

Caspase-8

Caspase-8 is a member of the caspase family of cysteine proteases, which are implicated in apoptosis and cytokine processing. Caspase-8 is a 55 kDa protein of 480 amino acids that comprises two death-effector-domains (DED) in its prodomain at the N-terminus and a C-terminal catalytic protease domain. The DED domain functions as platforms for protein-protein interaction (Barnchart et al. 2003)[57].

Caspase-8 is an initiator caspase that is present in most cells as proenzyme (Zymogen) in an inactive state. Upon the induction of apoptosis, caspase-8 becomes activated death activated via oligomerization in a multimeric complex at activated death receptors (Figure. 5) (Fulda 2009)[58]. Accordingly, the ligation of death receptors such as CD95 or the agonistic TRIAL-R1 and TRIAL-R2 by their corresponding ligands CD-95 ligand or trial or by crosslinking antibodies triggers receptor trimerization and clustering of the receptor death domains, which enables the recruitment of adaptor molecules such as Fas associated with a death domain (FADD) via hemophilic protein-protein interactions through the death domains (Ashkenazi 2008)[59]. Caspase -8 is in turn recruited to this complex via FADD through interaction of the DED domains, which leads to the formation of death- inducing signaling complex (DISC). This oligomerization of caspase-8 in the DISC drives its activation through autoproteolysis (Boatright and Salvesen 2003)[60]. Beside its proteolysis at the DISC, caspase 8 can also be activated downstream of mitochondria upon initiation of the intrinsic apoptosis pathways (Degterev et al., 2003)[56].

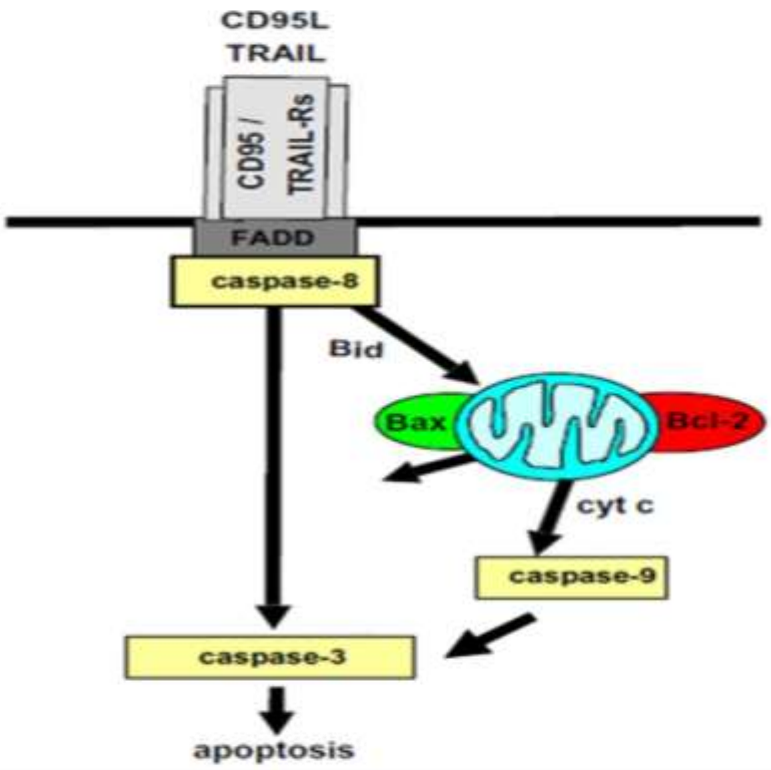

Figure 5: Apoptosis signaling pathways (Fulda 2009)[58].

Caspase 9

Caspase-9 is also an important member of the cystein aspartic acid protease (Caspase) family. Upon apoptotic stimulation cytochrome c released from mitochondria associates with procaspase-9 (47kDa)/Apaf 1. This complex process procaspase-9 into a large active subunit (35 kDa or 17kDa) and a small subunit (10 kDa) by self cleavage at Asp 315(Zou et al. 1999)[61]. Cleaved caspase 9 further process other caspase members, including cas-3 and cas-7, to initiate a caspase cascade leading to programmed cell death.

P53

P53 is a nuclear phosphoprotein of Molecular weight 53 kDa, encoded by a 20-kb gene containing 11 exons and 10 introns, which is located on the small arm of chromosome 17. Whereas, a wild-type P53 protein contains 393 aminocids and is composed of several structural and functional domains.

As a tumor suppressor, P53 is essential for preventing in-appropriate cell proliferation and maintaining genome integrity following genotoxic stress (Vousden and Lu 2002)[62]. P53 serves as multifunctional role as a transcriptional regulator, it mediates G1-S growth arrest and play a critical role in maintaining DNA integrity by facilitating apoptosis of DNA-damaged cells (Arun and Hortobagyi 2009)[63]. In addition, P53 mutations can lead either to loss or change of P53 binding activity to its downstream targets and may thus induce aberrant cell proliferation with consequent malignant cellular transformation. Based on P53's critical role in carcinogenesis, scientists have developed multiple effective strategies for treating cancer by enhancing function of wild-type p53 or increasing p53 stability.

Cyclin D1

The cyclin D1 proto-oncogene is an important regulator of G1 to S phase progression in many different cell types. Together with its binding partners cyclin dependent kinase 4 and 6 (CDK4 and CDK6), cyclin D1 form active complexes that promote cell cycle progression by phosphorylation and inactivating the retinoblastoma protein (RB) (Lundberg and Weinberg 1998)[64]. Phosphorylated pRB can no longer bind and repress the E2F transcription factors, which once liberated then proceed to activate genes that are essential for progression to the S-phase of the cell cycle. In addition to its CDK binding function, cyclin D1 can form physical associations with more than 30 transcription factors or transcriptional regulators and thus exert an important role in cellular growth, metabolism and differentiation (John 2007)[65].

Angiogenesis Markers

Angiogenesis is a normal and vital process in growth and development as well a in wound healing and in the formation of granulation tissue. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one by providing oxygen and nutrients to actively proliferating tumor cells.

VEGF

Vascular endothelial growth factor (VEGF) is a 34 to 42-kDa, dimeric, disulfide-bound glycoprotein. VEGF is an endothelium- specific factor, but is synthesized at a multiplicity of normal and pathological tissues sites, which include the stromal cells and infiltrating macrophages of the adipose tissues.

The presence of VEGF promotes the survival of the new vasculature by increasing the expression of the anti-apoptotic protein Bcl-2. High VEGF expression also promotes vascular permiablity *in vitro*, leading to high interstitial and intratumoral pressure, which may allow tumor cells to enter the blood stream and metastasis as well as impairing the delivery of chemotherapy to the tumor (Jain 2005)[66]. The VEGF ligands, which occur in several different splice variants and processed forms, have been identified so far. In certain respects, VEGFs share regulatory mechanisms with other well-characterized RTKs, such as the platelet-derived growth-factor receptors (PDGFRs) and the epidermal growth factor receptors (EGFRs). However, the VEGFRs also seem to be unique, for example, in their ability to transducer signals that form the three-dimensional vascular tube and in regulating vascular permeability that leads to the swelling of tissues. VEGFR1 is a positive regulator of monocyte and macrophage migration, and has been described as a positive and negative regulator of VEGFR2 signaling capacity. VEGFR2 is implicated in all aspects of normal and pathological vascular-endothelial-cell biology, whereas VEGFR3 is important for lymphatic endothelial-cell development and function. Several lines of evidence implicate the importance of VEGFA in breast cancer (Gasparini 2000)[67]. Patients with locoregional ductal cancers have elevated serum VEGFA concentrations in comparison with women with benign breast tumors. The highest concentrations of serum VEGFA were founded in metastatic breast cancer, in particular among patients who did not receive cancer therapy for metastatic disease (Salven et al. 1999)[68].

BRCA1 and BRCA2

The breast cancer susceptibility genes BRCA1 and BRCA2 encode multifunctional proteins, the mutant phenotypes of which predispose both to breast and to ovarian cancer (Wooster et al. 1995)[69]. BRCA1 and BRCA2 encode large nuclear proteins widely expressed in different tissues, markedly during S and G2 phase. They bear little resemblance to one another or to other protein of known function (Venkitaraman 2002)[70]. Both protein products have been consistently linked to various processes involved in the DNA damage response, acting as tumor suppressors. Understanding the normal functions and regulation of BRCA 1 and BRCA 2 may reveal how direct or indirect functional inactivation of BRCA genes ultimately leads to breast tumorigenesis. Late in 1999, an important series of experiments related the protein products of the *BRCA1* and *BRCA2* genes with the *ATM* protein kinase.

ATM homozygotes also show evidence of chromosomal instability and approximately a 100-fold increased risk for cancers. The ATM sequence bears a similarity to yeast genes that serve cell-cycle checkpoint and DNA repair functions. It was demonstrated that ATM phosphorylates *BRCA1*, activating a process of DNA repair through homologous recombination in cooperation with the *BRCA2* gene product (Cortez et al. 1999)[71]. Other proteins, including the product of *mRAD51*, and other molecules participate in this biochemical pathway (Figure. 6).

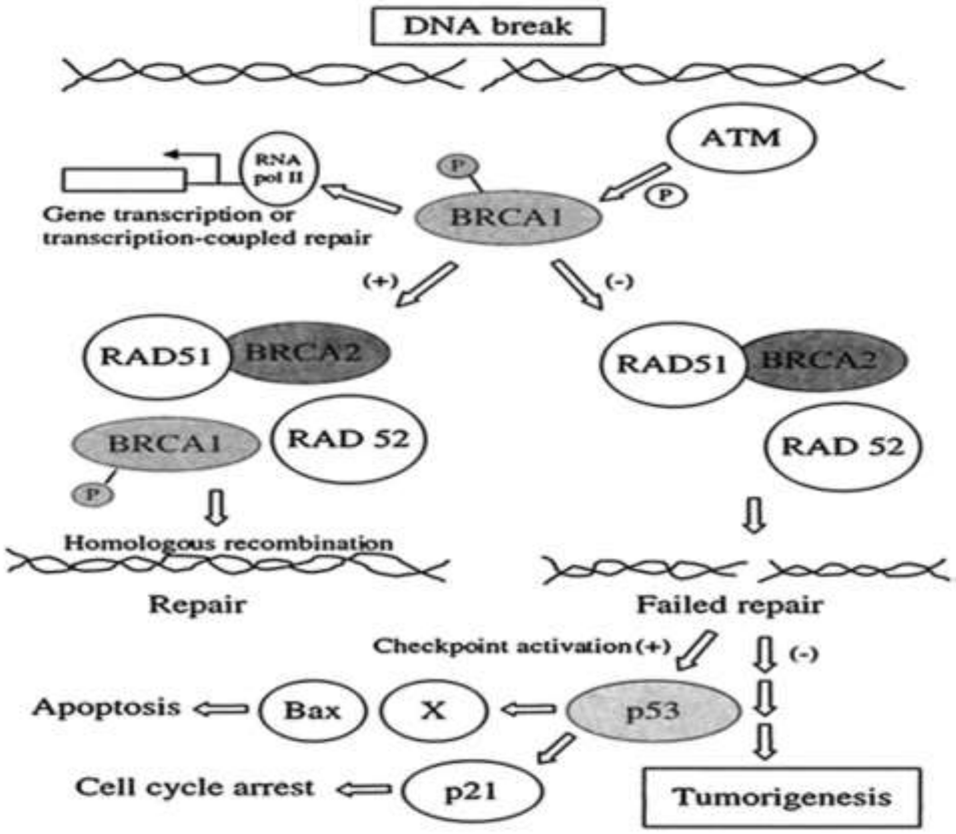

Figure 6. Current model for function of *BRCA* proteins (Kenneth offit 2000) [72].

ENZYMES

Aromatase Inhibitor

Aromatase, an enzyme of the cytochrome P-450 super family and the product of the *CYP19* gene, is expressed in several tissues, including subcutaneous fat, liver, muscle, brain, normal breast tissues, and mammary adenocarcinoma (Goss PE and Strasser 2001)[73]. The conversion of androgens to estrogens, the final step in estrogen synthesis, can be blocked by aromatase inhibitors. Aromatase activity, by increasing local estrogen synthesis, may play an early role in breast cancer carcinogenesis (Bulun et al. 1993)[74]. AIs are classified as first, second, or third generation according to the specificity and potency with which they inhibit the aromatase enzyme. They are further subclassified as type 1 or type 2 inhibitors, according to the reversibility of their inhibitory activity. Type 1 inhibitors, steroidal analogues of androstenedione, irreversibly inhibit the aromatase enzyme by covalently binding to it, thus earning the name “suicidal inhibitors.” Permanent inactivation persists after discontinuation of the drug until the peripheral tissues synthesize new enzymes. In contrast, nonsteroidal type 2 inhibitors bind reversibly to the aromatase enzyme, resulting in competitive inhibition(Goss PE and Strasser 2001)[73].

Third-generation AIs (i.e., anastrozole, letrozole, and exemestane) are the most potent, most selective, and least toxic AIs known today and can reduce serum estrogen by more than 95%.

In addition, their pharmacokinetic properties (a half-life of approximately 48 hours for anastrozole and letrozole and 27 hours for exemestane) allow for a once-daily dosing schedule (Wiseman and Adkins 1998)[75]. Their selective inhibitory properties allow their use without the need for supplemental corticosteroidal or mineralocorticoid supplementation, as is the case with the nonspecific AI aminoglutethimide.

Markers of tumor invasion and metastatic potential: MMP 13

One specific group of proteolytic enzymes, matrix metalloproteinases (MMPs), were studied extensively as key mediators of ECM degradation and in the processing of other bioactive molecules (Hua et al. 2011)[76]. MMPs also regulate cell surface growth factor "shedding" which regulates the proteolytic release of several proteins such as growth factors, chemokines and adhesion molecules. In a variety of different cancers, increased MMP expression and activation generally promote hallmarks of tumor progression including angiogenesis, invasion and metastasis, and correlate with shortened survival (Van der Jagt et al.2010)[77].

Proteins of the matrix metalloproteinase family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodelling, as well as in disease processes, such as arthritis and metastasis. Most MMPs are secreted as inactive pro proteins which are activated when cleaved by extracellular proteinases. The protein encoded by this gene cleaves type II collagen more efficiently than types I and III. It may be involved in articular cartilage turnover and cartilage pathophysiology associated with osteoarthritis. The gene is part of a cluster of MMP genes which localize to chromosome 11q22.3 (Tsai et al. 2014)[78].

Conclusion

An understanding the application of molecular and genetic marker in clinical oncology as prognostic factors and as potential targets for therapeutic intervention continues to evolve rapidly. They may well be useful in making decision regarding indentifying those who will benefit most and therefore avoid toxic side effects of treatment in breast cancer patients with the least risk for recurrence. Thus this review demonstrated the role of various putative biomarkers in breast cancer for early diagnostic benefit. Although we have covered a broad scope of the relevant literature, there are many studies that may have overlooked, and many important ongoing investigations with promise for the future.

REFERENCES

1. Hanahan D, Weinburg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646-674.
2. Kryston TB, Georgiev A, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutation Research 2011;711(1-2):193–201.

3. Cheang M, Vande Rijn M, Nielsen TO. Gene expression profiling of breast cancer. Annual review of pathology: Mechanism of Diseases 2008;3:67-97.
4. Giancotti V. Breast cancer marker. Cancer letters 2006;243(2):145-159.
5. Sawyers CL. The cancer biomarker problem. Nature 2008;452(7187):548-552.
6. Nowsheen S, Aziz K, Panayiotidis MI, Georgokilas AG. Molecular markers for cancer prognosis and treatment: Have we stuck gold?. Cancer letters 2012;327(1-2):142-152.
7. Hulka BS, Moorman PG. Breast cancer: hormones and other risk factors. Maturitas 2001;38(1):103-116.
8. Mitika M. Researches seek mammography alternatives. Journal of American Medical Associations 2003;290(4):450–451.
9. Qaseem A, Snow V, Sherif K, Aronson M, Weiss KB, Owens DK. Screening mammography for women 40 to 49 years of age: a clinical practice guideline from the American college of physicians. Annals of Internal Medicine 2007; 146(7): 511-5.
10. Roulston JE. Limitations of tumor markers in screening. The British Journal of Surgery 1990;77(9):961–2.
11. Grimm M, Schmitt S, Teriete P, Biegner T, Stenzl A, Hennenlotter J, Muhs HJ, Munz A, Nadtotschi T, König K, Sänger J, Feyen O, Hofmann H, Reinert S and Coy JF. A biomarker based detection and characterization of carcinomas exploiting two fundamental biophysical mechanisms in mammalian cells. BMC Cancer 2013;13:569.
12. Novakonic S. Tumor markers in clinical oncology. Radiology and Oncology 2004;38(2):73-83.
13. Clark GM, Dressler LG, Owens MA, Pounds G, Oldaker T, McGuire WL. Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. The New England Journal of Medicine 1989; 321(7):473-4.
14. Haslam SZ. The ontogeny of mouse mammary gland responsiveness to ovarian steroid hormones. Endocrinology 1989;125(5):2766-2772.
15. Tricbopoulous D, Mac Mahon B, Cole P. Menopause and breast cancer risk. Journal of National Cancer Institute 1972;48(3):605-613.
16. Speirs V, Skliris GP, Burdall SE, Carder PJ. Distinct expression patterns of ER alpha and ER beta in normal human mammary gland. The Journal of Clinical Pathology 2002;55(5):371-374.
17. Girdler F, Brotherick B. The estrogen receptors (ERα) and their role in breast cancer: a review. Breast 2002;9:194-200.

18. Carr BR, Macdonald PC, Simposon ER. The role of lipoproteins in the regulation of progesterone secretion by the human corpus luteum. Fertility and Sterility 1982;38(3):303-311.
19. Miller WL, Strauss JF. 3rd Molecular pathology and mechanism of action of the steroidogenic acute regulatory protein, StAR. The Journal of Steroid Biochemistry and Molecular Biology. 1999;69(1-6):131-141.
20. Anderson E, Clarke RB. Steroid receptor and cell cycle in normal mammary epithelium. Journal of Mammary Gland Biology and Neoplasia 2004;9(1):3-13.
21. Ismail PM, Amato P, Soyal SM, De Mayo FJ, Conneely OM, OMalley BW et al. Progestrone involvement in breast development and tumorigenesis as revealed by progesterone receptor “Knockout”and “knockin” mouse model. Steroids 2003;68(10-13): 779-787.
22. Shyamala G, Chou YC, Louie SG, Guzman RC, Smith GH, Nandi S. Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging. The Journal of Steroid Biochemistry and Molecular Biology 2002;80(2):137-148.
23. Bernstein L. Epidemiology of endocrine-related risk factors for breast cancer. Journal of Mammary Gland Biology Neoplasia 2002;7(1):3-15.
24. Payney RC, Allard JW, Anderson-Mauser L, Humphreys JD, Tenney YD, Morris DL. Automated assay for HER-2/neu in serum. Clinical Chemistry 2000;46(2):175-182.
25. Harari D, Yarden Y. Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. Oncogene 2000;19(53):6102-14.
26. Sliwakowshi MX, Lofgren JA, Lewins GD, Hotaling TE, Fendly BM, Fox JA. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). Seminar in Oncology 1999;26(4):60-70.
27. Shaks S. For the herceptin multinational study group. Seminar in Oncology 1999;26(4):71-7.
28. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. International Journal of Cancer 1983;31(1):13-20.
29. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. Journal of Clinical Oncology 2005;23(28):7212-20.
30. De Biasio A, Blanco FJ. Proliferating cell nuclear antigen structure and interactions: too many partners for one dancer?. Advances in Protein Chemisrty and Structural Biology 2013;91(1):1-36.

31. Lisa M, Coussens, Zena werb. Inflammation and cancer. Nature 2002;420(6917):860-867.
32. Muller U, Jongeneel CV, Nedospasov SA, Lindahl KF, Steinmetz M. Tumour necrosis factor and lymphotoxin genes map close to H-2D in the mouse major histocompatibility complex. Nature 1987;325(6101):265–267.
33. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. PNAS USA 1975;72(9):366-3670.
34. Goldberg JE, Schwertfeger KL. Proinflammatory cytokines in breast cancer: mechanisms of action and potential targets for therapeutics. Current Drug Targets 2010;11(9):1133-46.
35. Wu S, Boyer CM, Whitaker RS, Berchuck V, Wiener JR, Weinberg JB, et al. Tumor necrosis factor alpha as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor alpha expression. Cancer Research 1993;53(8):1939–1944.
36. Balkwell F. Tumor necrosis factor or tumor promoting factor?. Cytokine Growth factor revews 2002;13(2):135-141.
37. Storci G, Sansone P, Mari S, Dsuva G, Tavolari S, Guarnieri T et al. TNF alpha up-regulated SLUG via the NF-Kappa B/HIF1 alpha axia, which impact breast cancer cells with a stem cell-like phenotype. Journal of Cellular Physiology 2010;225(3):682-91.
38. Turini EM and Dubois RN. Cycloxygenase-2: A therapeutic target. Annual Reviews of Medicine 2002;53:35-57.
39. Davies G, Martin LA, Sacks N and Dowsett M. Cycloxygenase-2 (COX-2), aromatase and breasst cancer: apossible role of COX-2 inhibitors in breast cancer chemoprevention. Annels of Oncology 2002;13(5):669-678.
40. Sen R, Baltimore D. Multiple nuclear factor interact with the immunoglobulin enhancer sequences. Cell 1986; 46(5):705-716.
41. Junghan S, Arnold BR. NF-κB activation in human prostrate cancer: important mediator or epiphenomena?. Journal of Cellular Biochemistry 2004;91(1):100-17.
42. Rayet B, Gelinas C. Aberrant rel/nfkb gene and activity in human cancer. Oncogene 1999;18(49):6938-47.
43. Annunziata CM, Davis RE, Demchenko Y, Bellamy W, Gabrea A, Zhan F et al. Frequent engagement of the classical and alternative NF-Kappa B pathways by diverse genetic abnormalities in multiple myeloma. Cancer cell 2007;12(2):115-130.

44. Perkins ND. Achieving transcriptional specificity with NF-kappa B. The International Journal of Biochemistry and Cell Biology 1997;29(12):1433-1448.
45. Huber MA, Azoitei N, Baumann B, Grunert S, Sommer A, Pehamberger H, et al. NF-κB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. The Journal of Clinical Investigations 2004;114(4):569-581.
46. Silva MF, Khokhar AR, Qureshi MZ and Farooqi AA. Ionizing radiations induces apoptosis in TRIAL resistant cancer cells: *in vivo* and *in vitro* analysis. Asian Pacific Journal of Cancer Prevention 2014;15(5):1950-7.
47. Marzo I, Brenner C, Zamzami N, Jurgensmeir JM, Susin SA, Vieira HL, et al. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science 1998;281(5385):2027-2031.
48. Liu G, Wang T, Song J, Zhou Z. Effects of apoptosis-related proteins caspase-3, Bax and Bcl-2 on cerebral ischemia rats. Biomedical Reports 2013;1(6):861-867.
49. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993;74(4):609–619.
50. Cunha KS, Caruso AC, Faria PA, Silva LE, Fonseca EC, Geller M, et al. Evaluation of Bcl-2, Bcl-x and cleaved caspase-3 in malignant peripheral nerve sheath tumors and neurofibromas. Anaci Da Academia Brasileria Ciencias 2013; 85(4):1497-511.
51. Peter ME, Krammer PH. The CD95 (APO-1/Fas) DISC and beyond. Cell Death and Differentiation 2003;10(1):26-35.
52. Houston AM, Connell JO. The Fas signalling pathway and its role in the pathogenesis of cancer. Current Opinion in Pharmacology 2004;4(4):321-326.
53. Barnchart BC, Alappat EC, Peter ME. The CD 95 type I/ type II model. Seminars in Immunology 2003;15(3):185-193.
54. Nicholson DW. Caspase structure, proteolytic substrates and function during apoptotic cell death. Cell Death and Differentiation 1999;6(11):1028-42.
55. Khan Z, Bhadouria P, Gupta R, Bisen PS. Tumor control by manipulation of the human anti-apoptotic survivin gene. Current Cancer and Therapy Reviews 2006;2(1):73–79.
56. Degterev A, Boyce M, Yuan J. A decade caspases. Oncogene 2003;22(53):8543-8567.
57. Barnchart BC, Lee JC, Alappat EC, Peter ME. The death effector domain protein family. Oncogene 2003;22(53):8634-8644.

58. Fulda S. Caspase-8 in cancer biology and therapy. Cancer letter 2009;281(2):128-133.
59. Ashkenazi A. Targeting the extrinsic apoptosis pathway in cancer. Cytokine Growth Factor Reviews2008;19(3-4):325–331.
60. Boatright KM, Salvesen GS. Mechanisms of caspase activation. Current Opinion in Cell Biology 2003;15(6):725–731.
61. Zou H, Li Y, Liu X, Wang X. An APAF-1· Cytochrome C multimeric complex is a functional apoptosome that activates procaspase-9. The Journal of Biological Chemistry 1999;274(17):11549-11556.
62. Vousden KH, Lu X. Live or let die: the cell's response to p53. Nature Reviews Cancer 2002;2(8):594-604.
63. Arun B and Hortobagyi GN. Progress in breast cancer chemoprevention. Endocrine Related Cancer 2009;9(1):15-32.
64. Lundberg AS, Weinberg RA. Functional inactivation of the retinoblastoma protein requires sequential modification by at least two distinct cyclin-cdk complexes. Molecular and Cellular Biology 1998;18(2):753-76.
65. John PA. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Molecular Cancer 2007;6(24):1-16.
66. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307(5706):58−62.
67. Gasparini G. Prognostic value of vascular endothelial growth factor in breast cancer. Oncologist 2000;5(1):37-44.
68. Salven P, Perhoniemi V, Tykka H, Maenpaa H, Joensuu H. Serum VEGF levels in women with a benign breast tumor or breast cancer. Breast Cancer Research and Treatment 1999;53(2):161–166.
69. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Manqion J et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378(6559):789–792.
70. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 2002;108(2):171–182.
71. Cortez D, Wang Y, Qin J, Elledqe SJ et al. Requirement of ATM-dependent phosphorylation of Brca1 in the DNA damage response to double strand breaks. Science 1999;286(5442):1162-1166.
72. Kenneth offit. Are BRCA1 and BRCA2 assosiated breast cancer difference?. J Clin Oncol 2000;18(21):104-106.
73. Goss PE, Strasser K. Aromatase inhibitors in the treatment and prevention of breast cancer. Journal of Clinical Oncology 2001;19(3):881–894.

74. Bulun SE, Price TM, Aitken J, Mahendroo MS, Simpson ER. A link between breast cancer and local estrogen biosynthesis suggested by quantification of breast adipose tissue aromatase cytochrome P450 transcripts using competitive polymerase chain reaction after reverse transcription. Journal of Clinical Endocrinology and Metabolism 1993;77(2):1622–1628.
75. Wiseman LR, Adkins JC. Anastrozole: a review of its use in the management of postmenopausal women with advanced breast cancer. Drugs and Aging 1998;13(4):321–332.
76. Hua H, Li M, Luo T, Yin Y, Jiang Y. Matrix metalloproteinases in tumorigenesis: An evolving paradigm. Cellular and Molecular Life Science 2011;68(23):3853–3868.
77. Van der Jagt MF, Wobbes T, Strobbe LJ, Sweep FC, Span PN. Metalloproteinases and their regulators in colorectal cancer. Journal of Surgical Oncology 2010;101(3):259–269.
78. Tsai CF, Yeh WL, Chen JH, Lin C, Huang SS, Lu DY. Osthole suppresses the migratory ability of human glioblastoma multiforme cells via inhibition of focal adhesion kinase-mediated matrix metalloproteinase-13 expression. International Journal of Molecular Science 2014;15(3):3889-903.