Screening of Oncogenic Signaling Pathways and Therapeutic Application of Monoclonal Antibodies in Breast Cancer

[1#]Alka Patel, [2]Shivani, [3]Vinod Kumar Gupta

[1#]Project Trainee at Rapture Biotech International Pvt. Ltd., Noida (UP), India.
[2]Project Trainee at Rapture Biotech International Pvt. Ltd., Noida (UP), India.
[3]Technical Head (life sciences division) at Rapture Biotech International Pvt. Ltd., Noida (UP), India

***Abstract*: Breast cancer is one of the most common diagnosed cancers in the women worldwide. Due to the pervasiveness of breast cancer mortality rate increases. There has been a variety of factors involved in breast cancer progression such as pathological causes, role of different molecular and biochemical marker, genetic mutation, gene expression, cancer stem cells, signaling pathways, etc. Nowadays, breast cancer implication and developmental study is hot topic in clinical and basic research related to multiple cells surfaces and hormone receptor like Estrogens receptor , Progesterone receptor and Human epidermal growth factor receptor-2(HER2) and major signalling pathways such as Mitogen activated protein kinase (MAPK), calcium signaling, delta-notch, wnt/β-catenine signaling etc play an important role in it. Now clinical studies show that monoclonal antibodies opposed to specific protein receptor that involved in cancer growth and improve the immortality rate for women. In this review, crosstalk on inhibit the molecular mechanisms of specific signalling pathways through suppressed the over expression of the receptors via monoclonal antibodies that involved in breast cancer.**

***Keywords*: Breast cancer, Downstream signaling pathways, Monoclonal antibodies, Growth factor receptor.**

I. INTRODUCTION

Cancer is a disease in which cells are able to uncontrolled divided due to genetic changes in, particularly gene. Breast cancer is the most commonly diagnosed cancer in females, and one of the major causes of death seen in the world. Breast cancer is clinically and genetically heterogeneous groups of disease originated from the inner lining of epithelial cells of the ducts, and lobules that supply milk or the tissues. Breast cancer can occur in women but also occurs in men [1,2]. Male breast cancer is a rare disease that accounts for only 1% of all reported breast cancer [3]. A female breast is divided into 15-25 segments i.e., called lobes, each of these lobes has a lot of small glands called lobules created from grape-like clusters called alveoli. Alveoli are responsible for the production of milk. The lobes, and lobules are joined through a cylindrical tube called duct which helps in milk flow and reaches the nipples [4]. The breast consists of glandular tissues and stromal (supporting) tissues. Glandular tissues are milk-producing glands (lobules) and the milk passage (duct), stromal tissues include fatty and fibrous connective tissue of the breast. a breast is also composed of lymphatic tissue that removes cellular fluids and waste [5]. In breast cancer, the normal cells transform into a malignant cancerous cell due to over-expression of different types of genes acts as important role in breast cancer cell development [6]. The following steps are control of growth signals, strength of growth-inhibitory (anti-growth) signals, elusion of programmed cell death, unlimited reproductive capacity, prolonged angiogenesis, tissue attack, and metastasis [7]. Breast cancer is clinically divided into three types based on immunohistochemical analysis: luminal (estrogen receptor, α-positive), HER2 positive (human epidermal growth factor receptor2-positive) and basal-like. Every type has various risk factors, treatment response, disease progression risk and metastasizes sites of preferred organs. Luminal cancer is positive for estrogen receptors(ER) and progesterone receptors (PR) and responded to hormonal interventions, HER2 positive cancer have over-expression and amplification of ERBB2 oncogene and controlled with a number of anti-HER2 therapies. Basal-like cancer lack the expression of hormone receptors i.e, ERα, PR and HER2 so this is called triple-negative breast cancer (TNBC). there is no special molecular therapy for TNBC at that moment [8,9]. Thus the luminal and HER2+ve cancer are derived from luminal lineage progenitors while basal-like arises from less differentiated stem cells pattern of gene expression and evidence in the model system indicate that luminal progenitors can also act as a precursor to the epigenetic and genetic events in basal-like cancer [6]. Luminal is further categorized in luminal A and luminal B. luminal B expressed ER with HER2 and luminal A does not express HER2. HER2 is a member of the epidermal growth factor (HER/EGFR/ERBB) family, also known as ERBB2, CD340 or proto-oncogene neu. The tyrosine kinase protein receptor is situated on the plasma membrane of the cells. Tyrosine kinase activation facilitates the proliferation of the cells and inhibits apoptosis. After that HER2 can dimerize with any other ERBB family resulting autophosphorylation of tyrosine residues and activate the signaling pathways [10]. Some signaling pathways lead to breast cancer are a hedgehog, NOTCH, Wnt/β- catenin, GPCRetc. Ras/Raf/MEK/ERK and P13/AKT pathways are controlled by EGFR family of receptors [11]. Most of the breast cancer express estrogen(ER+), progesterone(PR+) receptors and human epidermal growth factor receptor(HER2+) treated with monoclonal antibodies tamoxifen for ER+ and PR+ and trastuzumab for HER2+ in breast cancer [12]. In 1998, trastuzumab is the first detected the over-expression of human epidermal growth factor receptor(HER2+) in breast cancer patients. Monoclonal antibodies, pertuzumab bind to the cell surface receptors and tyrosine kinase inhibitor that target intracellular pathways such as epidermal growth factor receptors and inhibits the kinase activity [13].

II. SYMPTOMS OF BREAST CANCER:

Breast cancer does not have any sign-in early stages, but as a tumor grows the common symptoms in the majority of women have diagnosed. Lumps that feel like a hard knot or a thickening in the breast or under the arm. Physical change in size and shape of nipple turned inward or a sore in the nipple areas. Blood is discharge from only one side of nipples, Skin irritation or changes (puckering, scaliness) occur. Warm, red swollen breast with or without rashes and pain in the breast [14].

III. CAUSES OF BREAST CANCER:

1. **Genetic risk factor**: the chances of breast cancer substantially increased in women's having either two inherited gene mutations such as BRCA1 and BRCA2. Women who carry BRCA1 mutated gene have 72% chances for breast cancer and the BRCA2 mutated gene has 69% chances for breast cancer. In men, the BRCA 2 gene mutation has a 6% risk for breast cancer relative to BRCA1 gene mutation throughout their lives [15].
2. **Gender:** more than 99% of all cases of breast cancer have been identified by women.
3. **Hormonal causes:** breast cancer may be caused by the changes in the hormonal stages, it could be early menstruation (before 12 years), late menopause (after 54 years), pregnancy in early-stage, hormonal replacement therapy (HRT), use of contraceptive pills.
4. **Other factors:** alcohol consumption, age, family history.

IV. STAGES AND CLASSIFICATION OF BREAST CANCER:

Stages is defines the location of cancer, how much the cancer has developed and whether it has spread. The stages of breast cancer are diagonised on TNM system(Tumor, Node, Metastasis). In this system breast cancer are followed by 5 stages i.e. stage0 (Non-invasive breast cancer) to stage 4(Metastatic breast cancer) [16]. Expert estimated that, B C can be invasive BC and noninvasive (insitu) BC. Invasive ductal carcinoma (IDC) is a common type of breast cancer which also known as infiltrating ductal carcinoma. It represents approximately 80% of invasive breast cancer in women and 90% in men. Invasive ductal carcinoma refers to cancer that has broken through the milk duct wall and begun to invade breast tissues. On the other hand, through the lymph nodes and bloodstream can be spread to the other parts of the body. Non invasive breast cancer can be classified into 2 major types such that Ductal Carcinoma in Situ (DCIS), lobular carcinoma in situ(LCIS) [17]. In situ ductal carcinoma (DCIS) is breast cancer which is recognize as discrete spaces filled with malignant cell usually recognized in basal cell layer composed of normal myoepithelial cell and known as "intraductal carcinoma". DCIS is considered "non-invasive" and "pre-cancerous" since it has not spread to any normal surrounding breast tissue beyond the milk duct. It has a zero stage and normally detected by screening mammography. DCIS covers a wide range of diseases from low-grade, non-life-threatening lesions to high-grade lesions. It was also categorized according to cell architectural patterns (solid, cribriform and papillary), tumor grade (high, moderate and low grade) and comedic histology presence or absence. Approximately 20-30% of those without diagnosis grow breast cancer. Lobular carcinoma in situ is also known as infiltrating lobular carcinoma which is a second common type of breast cancer. Around 10% of invasive breast cancers are lobular invasive carcinoma. Lobular carcinoma in situ refers to cancer that has burst through the lobule wall and has begun to penetrate breast tissues. LCIS are generally strongly positive estrogen receptors, making them highly sensitive to anti-hormonal drugs such as tamoxifen. In-situ cancer has high potential to become invasive cancer [18,19].

Triple-negative breast cancer is cancer that measures negative for receptors of estrogen, excess protein HER2 and progesterone receptors. Such result means that cancer growth is not stimulated by the hormones of estrogen and progesterone, or by the protein HER2. It is used as basal-like surrogate term; although, more detailed classification can provide better treatment guidance and better prognostic estimates [20].

V. TYPES OF SIGNALING PATHWAYS IN BREAST CANCER:

To understand the action mechanisms and biology of breast cancer have several signaling pathways that involved in cell growth and cell survival and control cellular and molecular properties of cells. Membrane receptors and ion channels receive the ligand i.e, hormones, neurotransmitters, antibodies, cytokines, ions and growth factors etc; from the extracellular surface that affect cell signaling. Activated the cellular receptors and ion channels bound with stimuli enhance the several signaling pathways (fig1) i.e. growth factor receptor tyrosine kinase(RTK), small GTPase(Ras), serine/threonine kinase(Raf/AKT), lipid kinase(P13Ks), nuclear receptor(ER) and other signaling pathways are hedgehog and NOTCH, (wnt)/β catenin, STAT, MAPKetc [21,22]. Inactivated tumor suppressor and activated protooncogenes are maintain cell cycle, cell growth and programmed cell death therefore mutation of such genes either the deletions or insertions can activate the different signaling pathways and loss or gain of their function initiate tumorigenesis in different types of cancer, included breast cancer [23].

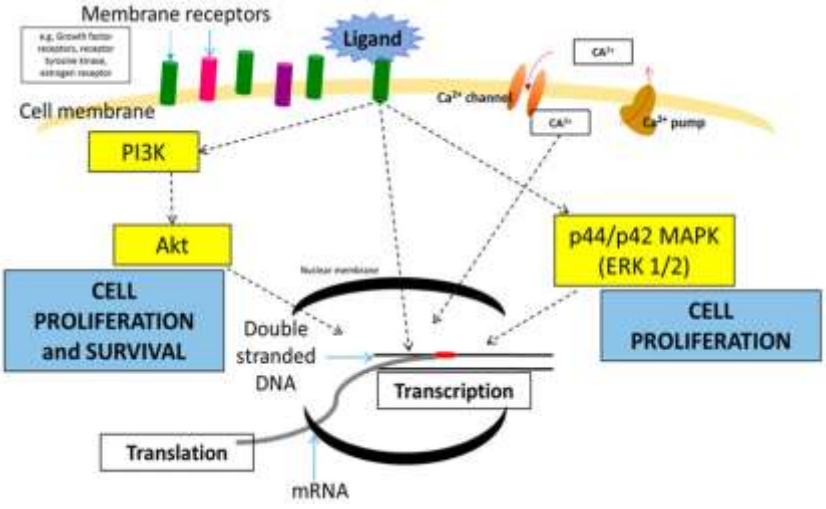

Fig 1: The crosstalk on the signaling pathways involved in breast cancer [23].

Calcium ion (Ca^{2+}) signaling pathways

In breast cancer, increases of intracellular Ca2+ ion may be an essential role for cell proliferation, differentiation, apoptosis, cytotoxicity, metabolisms, and gene transcriptions as well as leading to the mechanisms of anti-cancer agents [24,25]. Ca2+ ion is the most important and second messenger molecules for cellular signaling and cannot be metabolized, so cell monitor intracellular Ca2+ levels through a specific extrusion and binding proteins. The concentration level of intracellular Ca2+ ion in mammalian cells is approx 100mM and the concentration of extracellular Ca2+ levels is approx 2mM [26]. Ca2+ ion is ubiquitous in nature that phosphorylated the contractile proteins or activates the Metalloprotease and plays a significant role in the remodeling of peripheral protein and cell-matrix adhesion during cancer cell migration [27,28] and changes the shape and charge of the proteins that interact with particular components. Increasing in cytosolic Ca2+ ion calcium enter from the plasma membrane or Endoplasmic reticulum (ER) through ion gated channels and degraded inositol 1,4,5, triphosphate(IP3) receptors are enhanced by the phosphatase and tensin homolog(PTEN) protein [29]. Calcium ion triggers the Cyclin-dependent kinases (CDK4/CDK2) to enhance the G1 to S phase of the cell cycle [26]. Overexpression of highly selected plasma membrane calcium channels, transient receptor potential vanilloid-6 (TRPV6) in breast cancer decreases the basal calcium influx and cellular proliferation through altering the TRPV6 expression [30]. Noted that, invasiveness of breast cancer diminished by mute over expression of TPV4 [31]. Currently, the study shows that Rap2B is a GTP binding protein that enhances the intracellular calcium level by phosphorylation of extracellular signal-related kinase(ERK)1/2 which inhibits MEK1/2, BCap-37, and MDA-MB-231 breast cancer cells [32]. Calcium production from Endoplasmic reticulum(ER) and mitochondrial absorption causes programmed cell death. To survive cancer cells must be required a high concentration of reactive oxygen species(ROS). The transcription factor NRF2 regulates the function of the transient receptor potential ankyrin1(TRPA1) channel (fig2) [30]. ROS is generated by mitochondria after that hydrogen peroxide (H2O2) must be oxidized cysteine residues on TRPA1 to trigger the protein. As a consequence, the concentration of intracellular calcium ion raise, triggering a cascade of MCL1 protein involved in calcium-dependent anti-apoptotic and pro-survival signaling, therefore, ROS can encourage the survival of breast cancer cells by calcium signaling [33].

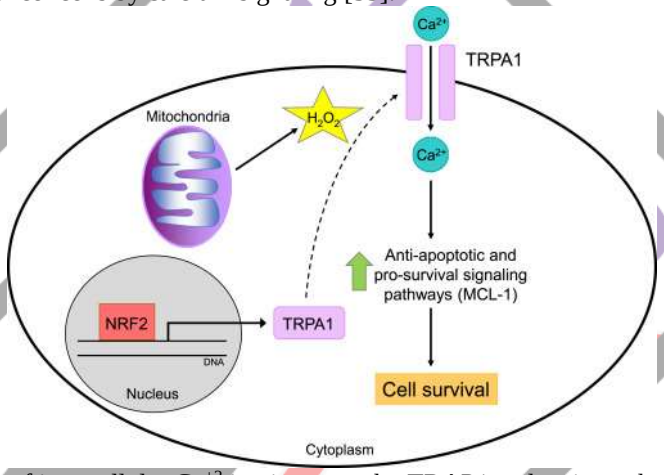

Fig 2: Concentration of intracellular Ca^{+2} ion increases by TRAP1 and activate the MCL1 protein [30].

Mitogen activated protein kinase (MAPK) signaling pathway

MAPK is the proteins that control a lot of signaling pathways to promotes many cellular programs, such as cell movement, cell differentiation, cell proliferation, cell division, embryogenesis and cell death in breast cancer [34,35]. MAPK cascades are biologically preserved in all eukaryotes and play a significant role in the initiation, progression and gene expression of breast cancer by transmitting extracellular impulse to intracellular stimuli. MAPK cascade is usually grouped into a 3 kinase structure composed of a MAPK, a MAPK activator(MEK, MKK, MAPK kinase) and a MEK kinase activator(MEKK kinase [MEKKK], MAPK kinase). In MAPKs, 6 diverse groups analyzed, such as extracellular signal-regulated kinase(ERK)1/2, controls cell growth and differentiation, ERK3/4, ERK5, ERK7/8, Nemo like kinase(NLK), C-Jun N- terminal kinase(JNK) and p38(α, β, γ, δ) MAPK cascades response in inflammation and apoptosis [36,37]. A small GTP binding protein of the dual specific kinase(Ras/Rho) family hydrolyzed MAPKKK. Activation of MAPKKK phosphorylated Serine-Threonine kinase and activate MEK via binding of the ligands with extracellular receptors that conserved kinase domain subdomain VIII. Simultaneously phosphorylation of tyrosine and threonine are triggered MAPKs that conserved threonine-X tyrosine sequence. Due to partially block of MAPK signalling pathways can be done proliferation, apoptosis, cell cycle arrest in the G2/M phase, innate and acquired immunity, cell repair [37,38,39]. Metastatic cells in neoplasm breast cancer show the hyperexpression of MAPK [40]. MEK enzyme is a MAPK pathway component that is inhibited by AZD6244(ARRY-142866) that's recently involved in breast cancer of phase 1 clinical stages [41]. Reduced the expression of MAPK showed diminished propagation and migration of basal-like breast cancer [39].

Delta Notch signaling pathways

The delta notch signaling pathways(DNSP) is that the conserved evolutionary pathway, first observed in Drosophila at the beginning of the 20th century by genetic mutations and in mammals involved in breast cancer growth. Notch signals arise from two mechanisms: CSL dependent signals (CBP/RBP- jk in vertebrates, Drosophila's hairless suppressor and Caenorhabditis Elegans' Lag 1) and Deltex protein signals. Delta notch gene is a single pass heterodimeric transmembrane protein that interacts via cell to cell signaling through delta-serrate, Lag-2(DSL) [42,43]. 4 notch receptors in a human being are notch1/TAN1, notch2, notch 3,

notch4/Int3 and transmembrane like DSL ligands contains of 5 classes are Jagged1, Jagged2, Delta like-1, Delta like-3, Delta like-4 [44]. The notch receptor is cleavages by the signal receiving cells and glycosylation of Ca2+ ions within the ER and Golgi [45]. DSL ligands attach to the extracellular domain's epidermal growth factor receptor(EGFR) and induce receptor cleavage i.e. regulated by ADAM family proteases and γ-secretase that removes the notch intracellular domain(NICD) into the nucleus and generated a tri-protein activation complex that interacts with DNA bound protein, CBF1/RBP-jk/Su(H)/Lag1 [46]/ CSL to induce gene 44, Based on specific ubiquitination and proteasomal degradation of NUMB protein which over expressed Hey1 and abundance of NICD increased the Notch signaling, especially in breast cancer [42,43]. Notch signaling to be identified with specific subtypes of breast cancer treatment triggers the ERα+ve to activate notch signaling. Increases expression of notch 1 and notch 4 observed in triple negative basal-like and HER 2 positive. Hyperexpression of notch 2 was correlated with highly differentiated and infrequently proliferative in carcinoma. It had been observed in usual ductal hyperplasia (UDH), ductal carcinoma insitu (DCIS) and pre-invasive carcinoma. Finally, a new study found that a sequence of mutation in notch1, notch2, and notch3's pest domain enhance triple negative breast cancer [47]. Introcancerous cells of 73% to 100% in breast adenocarcinomas and 18% in ductal carcinoma in situ are generated by DII-4. Different types of cellular processes such as proliferation, hypoxia, angiogenesis, apoptosis, cancer stem cell function, metastasis, epithelial to mesenchymal transition (EMT) are also controlled by notch signaling. The breast epithelial cells are protected from apoptosis by triggering AKT pathways and facilitate cell proliferation in breast cancer by increasing the expression of cyclinA, cyclinB, cyclinD1, through notch signaling [44,47].

Hedgehog signaling pathway

Hedgehog protein was first identified in Drosophila Melanogaster. In vertebrate animals, three different types of homologous hedgehog proteins are sonic hedgehog (SHH), desert hedgehog (DHH), Indian hedgehog (IHH) [48]. All active forms of hedgehog protein are initiated by many processes during the development and maintenance of mammary glands, cell proliferation, apoptosis, differentiation, regeneration, homeostasis, and embryogenesis [48,49]. Three important transmembrane proteins involved in this signaling is Patched, Smoothened, and GLi(glioma-associated oncogene) [50]. GLi is a family of zinc finger motif [51]. Hedgehog signaling is activated by covalently attached hedgehog ligands to its patched receptor(Ptch 1) therefore this is lipid-associated ligands [48]. In presence of hedgehog protein Patched is a 12 transmembrane helical protein that catalyzed the 7 transmembrane helical protein, Smoothened present in intracellular vesicles. It regulates and phosphorylated the smoothened later inactivation of patched Ci155 translocate into nucleus and induce siganiling, hyperexpression of hedgehog signaling causes cancer in mammary glands [42,51,52]. Ptch was lost in 58% of invasive carcinomas, it describes a wide range of pathways that can include the hedgehog signaling in the carcinogenesis are cell cycle regulation (Cyclin D1/2), proliferation(PDGFR, MYC), apoptosis (BCL2), angiogenesis (VEGF, ANG1/2), epithelial-mesenchymal transition [EMT] (MMP9, SAAIL), self-renewal (NANOG, SOX2) and genes in feedback mechanisms(HHIP, Ptch1, Gli1) [53]. Hyeperexpression of GLi promotes memory cancer in mammals [54].

Wnt/β-catenine signaling pathways

Wnt signaling was discovered in the site of the mouse mammary tumor virus(MMTV) and in Drosophila Wingless gene(Dint 1) [55]. It is evolutionarily conserved pathways that play a crucial role in cell-cell adhesion, proliferation [56], cell migration, cell polarity, organogenesis , and cell fate determination. Based on downstream of Frizzled receptor(Fz), three important pathways involved in this signaling is a canonical or Wnt/β-catenin dependent pathway, the non-canonical or Wnt/β-catenin independent pathway which can be a divided on the basis of planer Wnt/Ca2+ pathway [57] and cell polarity pathway [58]. For breast cancer, autocrine signaling triggers the regulation of the Wnt pathway [22]. Mammals have 19 Wnt genes in which Wnts 1,2,3A,5B,7A,7B expressed in human breast tissues. Canonical Wnt signaling is presence of the Wnt that bind to Fz receptor, seven trans-membrane helical protein [58,59] and single transmembrane low-density lipoproteins5/6(LPR5/6) co-receptors activate Dishevelled protein(Dvi) that phosphorylates the β-catenin-axin-APCcomplex and enhance the cytoplasmic level of β-catenin [42,60,61] by low phosphorylation and ubiquitination, because of that β-catenin enter into the nucleus [42]. These genes also upregulated the mesenchymal stem-like triple-negative breast cancer and basal-like 2 triple-negative breast cancer [62]. Excessive Wnt/β-catenin signaling causes breast cancer in human by mutation and over expression of receptors(LRP6, FZD7), transducers(Dvl 1), canonical Wnt protein(Wnt1, Wnt 10) and under expression of non-canonical Wnt protein (Wnt5a, Wnt 5b) and negative transducers(APC) [63].

JAK-STAT signaling pathways

JAK (Janus kinase) are intracellular protein that associated with receptor tyrosine kinase enzyme and signal transducers and activator of transcription (STAT) protein act as important transcription factor [64]. In this pathway, extracellular stimulli such as cytokines, interleukins, and growth factor are responsible for activating JAK/STAT signalling pathway [65]. In humans, 4 possible JAK family protein JAK1,JAK2, JAK3 and TYK2 and 7 STAT members of family protein STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6 have been identified [66]. JAK/STAT signalling plays a major role in proliferation, differentiation, apoptosis [67], angiogenesis [68], migration [69], tumor growth, hematopoiesis and inflammatory response [70]. JAK phosphorylates tyrosine residues at the C-terminal cytoplasmic end of STAT through SH2 domains. Undergoes phosphorylation of STAT form hetrodimer(STAT-STAT) and active dimer translocate into the nucleus, where they identified specific DNA region to activate or repress transcription [71]. Activation of JAK/STAT signaling pathways is control by negative feedback loop, suppressor of cytokine signaling (SOCS) protein that act as competitive inhibitors to STAT. While STAT is positively regulate transcription of SOCS gene [70]. Study showed that, STAT3 is strongly promoting the tumor growth in human. Over-activation of STAT3/STAT4 reported in many cancer included breast cancer [68] which phosphorylate the Tyr^{705} residues [72].

PI3K/AKT signaling pathways

The phosphatidylinostol-3-kinase (PI3K)/AKT are the intracellular signaling pathway that involved in various cellular process such as proliferation, cell growth, tumor development [73,74], metabolism, motility, genomic stability, angiogenesis [75], apoptosis and cytoskeletal rearrangement [76]. In humans, the PI3K/AKT pathway is a very sophisticated signaling pathway that plays an

important role in breast cancer cell growth [77]. In this pathways, the 3 main component are PI3K (a lipid kinases family), a phosphatase and tensin homolog (PTEN) (tumor suppressor) and mTOR-the serine/ threonine protein kinase (effector of PI3K and AKT) [78]. AKT expressed 3 different isoforms in human also belongs to PKB family i.e. AKT1 (PKBα), AKT2 (PKBβ), AKT3 (PKBgamma) [79] which has similar structure and different function [80]. Class 1A, PI3Kinases are the hetrodimers which activated by the varieties of extracellular stimulli such as growth factor, cytokines and hormones binds to intracellular receptor tyrosine kinase(RTK). Upon activation of PI3Ks converts phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-triphosphate (PIP3)[81]. PIP3 kinases act as a docking site for protein contain Pleckstrin Homology (PH) domain i.e. PDK1 and AKT interact with the inner leaflet of plasmamembrane. Monophosphorylated AKT translocate into cytosol and also phosphorylate the two conserved residues, thr^{308} and ser^{473}. After both phosphorylation Akt becomes activated [74,75,76] and promote the positive regulator for cell survival, cell proliferation [82]. Due to overexpression of phosphorylated AKT protein was reported in 33% of DCIS and 38% of invasive cancer [83]. PTEN expressed as a negative or positive feedback regulator by activation of mTORC1 and S6K1. S6K1 is able to phosphorylate IRS-1 at multiple serine residues, control binding to RTKs, that suppress the activation of PI3K therefore PTEN acts as a tumor suppressor (Fig3). Mutation in PTEN protein increases the rate of human invasive breast cancer [83,84].

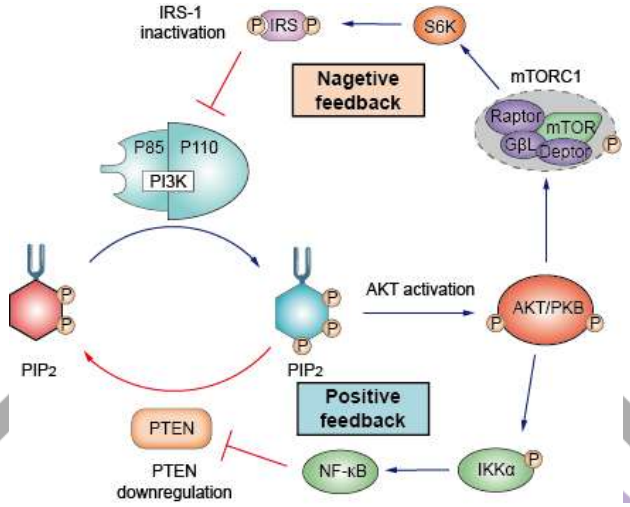

Fig 3: PTEN act as the positive feedback inhibitors in PIP2 in human breast cancer [85].

VI. MONOCLONAL ANTIBODIES (mAbs) INHIBITED THE BREAST CANCER SIGNALING PATHWAYS BY TARGETING GROWTH FACTOR RECEPTORS

Antibodies produced from clone of single lymphocytes against single epitope rather than a whole epitope this antibodies is called monoclonal antibodies(mAbs). mAbs are produced by Hybridoma technology. The production of mAbs was invented by Cesar Milstein and Georges J. F. Kohler in 1975 and they got the Nobel prize in 1984 [86]. Several humanized or chimeric mAbs have been approved by the FDA. These mAbs are used to target and kill cancer cells. The growth factor receptor protein engaged in cell signaling pathways that regulate cell division and survival. In some cases, Growth factor receptor gene mutations lead to increased cancer cell [87]. We focused here some human mAbs that was produced using transgenic mice. Human immunoglobulin genes have been inserted into the mice genome and the transgenic mice is vaccinated against the targeted antigen resulting in the development of human mAbs. Fully human mAbs, immunogenicity is very poor because of 100% of human origin [88]. Monoclonal antibodies target growth factor receptors in treatment of breast cancer has been approved one of the most effective therapeutic approaches for both hematologic malignancies and solid tumors. Monoclonal antibody binds to the specific targets cells and promotes patient's immune system to attack those cells or inhibits tumor growth [89]. Monoclonal antibodies can be antagonistic to cell signaling pathways by binding and blocking specific target cells surface receptor that prevent receptors engaged with signaling factor, neutralization of soluble signaling factor(e.g. VEGF, HER etc) and down the expression of cell surface receptors. Most interesting action of monoclonal antibodies reduced the expression of receptors by accelerating the internalization and catabolism of receptors. Both the direct antigen binding (Fab) and constant (Fc) domains of mAbs can contribute to their biological activities [90]. In most of the cases, blockade of cell signaling will not require involvement of Fc domain of monoclonal antibodies [91]. The half life of the mAbs is 3-4 weeks and because of their size it cannot cross the blood- brain barriers [92].

Anti-Human epidermal growth factor receptor (HER) mAbs

Human epidermal growth factor receptor-2 is a member of four membrane tyrosine kinases and play an important role in many cellular processes such as cell proliferation, differentiation, survival and migration [93,94]. Due to over expression of HER2 protein, 20-30% breast cancer is reported. The HER2 gene lies on the long arm of chromosome 17 and encodes 185KDa trans-membrane protein [95]. The plasma-membrane has HER2 receptor activated by ligand binding to extracellular domain and allows the formation of homodimers/ hetrodimers [96]. The HER 2 does not have identifiable ligand. Dimerization of such receptors leads to phosphorylates tyrosine residues within the cytoplasmic domain. This residues act as binding sites for adoptor proteins that contains Src homology 2 and phosphotyrosine binding domains (PTB) [94]. The first humanized IgG1 monoclonal antibodies (mAbs) approved by FDA in 1998 for breast cancer therapy are Trastuzumab (Herceptin) in phase II and III trails [90]. The clinical studies showed that, the Trastuzumab is an anti-HER2 mAbs approved for treatment of metastic breast cancer [97,98]. It was first biological drug approved for the treatment of HER2 positive breast cancer. The half life of Trastuzumab observed from 1.1days (10mg dose) to 23 days (500mg dose) [99]. It is binds to the amino acids present on domain IV of HER2 extracellular domain and these proteins

inhibits many downstream signaling pathways i.e. MAPK, PI3K/AKT, JAK/STAT signaling pathways. Pertuzumab (perjeta) is also a humanized MAbs that approved in 2012 for HER2 positive breast cancer. Firstly it inhibits the HER2 signaling without ligand activated and secondly stop the hetrodimerization of any EGFRs family [71].

Anti-Vascular endothelial growth factor receptor (VEGFR) mAbs

VEGFR is a transmembrane tyrosine kinase receptors which contains seven immunoglobulin like domains that play a essential role in angiogenesis and metastatic growth in human carcinogenesis [100,101]. VEGFR family in humans contains 3 members i.e.VEGFR-1(Flt-1) [102], VEGFR-2(KDR/FIK-1 in mice) [101], VEGFR-3(Flt-4) abundantly expressed in endothelial cells [103]. Among of these 3 receptors VEGFR-2 have a principle role in VEGFR in humans [104]. VEGFR-2 confirmed as suppressor of tumoreginesis in human breast cancer by small compound YLL545 [105], and isomangiferin [106]. Autophosphorylation of Tyr^{1175} in VEGFR-2 initiates downstream signaling and activates MAPK/ ERK1/2 and STAT3 that also plays a crucial role in tumor growth [105]. Clinical studies show, Bevacizumab(Avastin), the humanized monoclonal antibodies approved in 2004 can reduce tumor angiogenesis and inhibit the growth of solid tumors, either alone orin combination with vinorelbine produced inspiring result in phase II metastatic breast cancer [107]. It is also a humanized IgG1 mAb that binds to VEGF and prevent to bind the VEGF receptor on cancerous cells [108].

Anti-Epidermal growth factor receptor (EGFR) mAbs

EGFR is 170kDa transmembrane glycoprotein tyrosine kinase growth factor that identified in triple negative breast cancer (TNBC) and inflammatory breast cancer(IBC) [109]. It was the first growth factor that discovered by Stanley Cohen in 1962 from new born mice [110]. The EGFR family (also knowns as ErbB) has four types of receptors i.e. ErbB1/HER1, ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4 which contains extracellular ligand binding domain and intracellular tyrosine kinase domain that activated by homodimerization or hetrodimerization of autophosphorylated tyrosine residues allow the receptor to bind with ligand and overexpressed the downstream signaling i.e. MAPK, PI3K/Akt, Ras/Raf and JAK/STAT pathway. Non clinical studies revealed that the Panitumumab (Vectibix), is a human IgG2 kappa mAbs that bind to the EGFR1 and autophosphorylates and activates the kinases which inhibits the cell growth,apoptosis in breast cancer. It was first approved in 2006 by FDA for treatment of EGFR-positive metastatic colorectal carcinoma. On the combination of Panitumumab and Cetuximab with taxane-anthracycline-containing regimens inhibits TNBC as greater efficiency compared to each treatment. In clinical testing, IMC-C225, a chimeric monoclonal antibody; EMD 55900 (MAbs 425), a murine anti-EGFR monoclonal antibody; ICR 62, a rat monoclonal antibody; and ABX-EGF, a fully human anti-EGFR antibody. IMCC225 is an anti-EGFR monoclonal antibody that currently is in Phase II and Phase III testing inhibits the EGFR 1 [111,112,113]. Such adverse effects are generally associated with the antigens they are targeting and the intravenous mechanism of action in patients is rashes, low blood pressure and flu like symptoms. For example: Bevacizumab, inhibits the development of blood vessels in tumors and induces adverse effects such as hypertension and kidney damage [114]. In the use of Trastuzumab either alone are combination with chemotherapy can lead to heart failure, leucopenia, anemia, diarrhea, abdominal pain, and infections [115].

VII. CONCLUSION

Signaling pathways in breast cancer and other cancer are somatically disrupted at different frequencies and in different combinations across various organs and tissues, suggesting complex interaction and crosstalk pathways. Knowning the magnitude, complexity and co-occurrence of oncogenic changes in these pathways are crucial to the development of new therapeutic strategies that can benefit to patients care. The diverse oncogenic genetic varients in breast cancer signaling pathways are viable for therapeutic targets. The concept of using mAbs for cancer therapy is one of the significant contributions of tumor immunology in cancer patients. This achievement is based on the scientific research at the serological characterization of cancer cells, techniques for the generation of antibodies to target tumor, through analysis of signaling pathways specific to cancer cells and understanding of typical relationship of breast cancer cells with the immune system. The clinical production of mAbs is intertwind to the systematic and through study of the effects of in vivo antibodies and the evaluation of functional effects on cancerous cells but the chemotherapy as cancer treatment stimulates various side effects that are unbearable for the patients.

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