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BREAST CANCER: INSIGHTS INTO THE MAJOR SIGNALING PATHWAYS IMPLICATED IN MOLECULAR PATHOGENESIS

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Abstract:

As the most commonly occurring cancer in women worldwide, breast cancer poses a formidable public health challenge on a global scale. Breast cancer encompasses of a group of biologically and molecularly heterogeneous diseases originated from the breast. Regarding gene profile studies, breast cancer can be categorized, according to the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor (HER)-2 expression, into five molecular subtypes: luminal A, luminal B, HER2-overexpressing, triple negative and normal-like breast cancer. ER pathway plays prominent roles in development and progression of carcinogenesis by preventing apoptosis especially in ER+ breast cancer. In addition, breast cancer proliferation is enhanced by epidermal growth factor (EGF), which stimulates signaling through the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways by activation of the epidermal growth factor receptor (EGFR) axes. These pathways are tightly interconnected with ER-activated signaling and multiple points of cross talks are now elucidated. The latent relevance of ER, PI3K/AKT/mTOR and Raf/ MEK/ERK signaling pathways to all major subtypes of breast cancer will be discussed in this review.

Keywords: Breast cancer; Estrogen receptors; HER-2; PI3K/AKT/mTOR; RAS/RAF/MEK/ERK.

Introduction:

As the foremost common cancers in females worldwide, breast cancer poses a major health challenge. All over the world, more than 1.5 million breast cancer females are diagnosed annually. Being a multi-step process, multiple cell types are involved in the progression of breast cancer. The prevention of breast cancer remains challenging and early diagnosis of breast cancer, and other cancers, is one of the best attempts to prevent the progression of this disease. Metastatic breast cancer can frequently migrate to remote organs such as liver, brain, lung and bones, which principally accounts for its incurability [1]. Several desegregated signaling pathways are involved in breast cancer as they consequently bearing cell survival, proliferation, migration, differentiation, and apoptosis [2]. In this review, we have focused on Estrogen Receptor (ER) signaling, Epidermal Growth Factor Receptor (HER/EGFR) and other two major signaling pathways modulated by EGFR, the RAS/Raf/ mitogen-activated and extracellular signal-regulated kinase (MEK) /extracellular signal-regulated kinase (ERK) and the phosphatidylinositol 3-kinase (PI3K)/AKT/ mammalian target of rapamycin (mTOR) pathways. The latent relevance of PI3K/AKT/mTOR and RAS/RAF/MEK/ERK signaling pathways to all major subtypes of breast cancer will be discussed.

Breast cancer comprehends diverse biological subtypes which have discrete manners and responses toward therapy and that diverge extensively in disease prognosis.A number of intrinsic genes that distinguish these subtypes have been revealed. These genes are formed of multiple collections of genes associated with ER expression, an exclusive cluster of genes named the basal cluster and human epidermal growth factor 2 (HER2) expression Through a utilization of these sympathies, breast cancer can be categorized into five molecular subtypes depending on the expression of certain genes[3].

1. Epidermal Growth factor receptor:

Apart from ER-mediated progression, Breast cancer cells utilizes another pathway to execute their pathological behavior. Among them, growth factor receptor-mediating signaling is the dominant one. It has been demonstrated that HER signaling integrates with ER signaling and contribute to endocrine resistance [4].The family of HERs encompasses 4 receptor tyrosine kinases (RTK) (1-4) that are expressed not only in normal tissues but also in various types of tumors. The prominent HER-2, a member of the EGFRs, is a RTK that comprises three domains: an intracellular domain, a transmembrane domain, and an extracellular ligand-binding domain[5]. HER-2 regulates various cellular physiological and pathological functions through multiple pathways as the constitutively active form makes HER2 the preferred constituent to form dimers with other molecules [3]. Upon ligand binding and dimer

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formation, HER-2 is phosphorylated at its tyrosine residue of the extracellular domain. The phosphorylated HER-2, the active HER-2, transduces signals via multiple downstream signaling pathways such as the MAPK and PI3K pathways. These pathways recruit the progression of various malignant tumors by modulating the growth, cell cycle, reorganization of cytoskeleton, apoptosis, migration, invasiveness, differentiation, angiogenesis and transcription of various genes[6-9]

It is becoming increasingly evident that the carcinogenic process captures the pathways regulating normal cellular physiology such as PI3K/AKT/mTOR and the RAS/RAF/MEK/ERK cascades and uses them to transduce constitutively active survival signals to the nucleus. Furthermore, the PI3K/AKT/mTOR pathway modulates cellular energy, senescence and angiogenesis, and promotes the transcriptional activity of ER in ER-positive breast cancer. Numerous points of junction and feedback loops have been recognized lately and the bidirectional crosstalk between ER and GFR pathways adds marvelous complexity to the cell signaling network [10].

Activation of HER-2 expression or gene amplification triggers ER signaling [4], so HER2-targeted therapies are used in combination with endocrine therapy that blocks ER activity to treat this subtype. Activated ER pathway can provide substituted proliferation and survival signaling that could allow escape from efficient HER2 inhibition. Moreover, it has been revealed that the expression of HER family members is often amplified in cancers that have experienced endocrine resistance, leading to augmented signaling of this pathway and its downstream mediators[11].

1.1. The role of PI3K/AKT/mTOR pathway in directing breast cancer:

The PI3K/AKT/mTOR signaling pathway participates in various cell processes, including expansion, development, and endurance. Upon RTKs and G-protein-coupled receptor activation, a family of signaling enzymes referred as PI3K proteins are triggered.Three classes of lipid kinases are included in the PI3K protein family. These kinases catalyze the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). Among the various classes of PI3K, Class IA PI3Ks are composed of a catalytic and a regulatory subunit arranged in heterodimers. It has shown that this class of PI3K is the most commonly conveyed in human cancer [16].

In response to PI3K activation, the essential transmitter of cellular signaling PIP3 can transduce signals to trigger downstream signaling mediators, including phosphoinositide-dependent kinase 1 (PDK1) and AKT leading to activation of serious of signaling events that regulate cell survival and proliferation. At the plasma membrane PDK1 binds PIP3 which facilitates phosphorylation of AKT. Phosphorylated AKT can phosphorylate numerous effectors and targets and can translocate to the nucleus, thereby recruiting proliferation, cellular growth and angiogenesis, and inhibiting apoptosis [17].

Activated AKT can negatively regulate the tuberous sclerosis complex (TSC): TSC1 and TSC2, and thus triggers mTORC1 complex, the recruiter of cellular progression and protein synthesis that involves mTOR. Another mechanism by which AKT can mediate cancer survival is by phosphorylating Mdm2, a protein that hinders the stimulation of the tumor suppressor protein (p53) as shown in (Figure 1). In addition, AKT may suppress BAX, BAD, (the pro-apoptotic Bcl-2 family members) and forkhead transcription factors including FOXO. Phosphatase and tensin homolog (PTEN) can stimulates the dephosphorylation of PIP3 and thus antagonize the enzymatic activity of PI3K. Also inositol polyphosphate-4- phosphatase type II (INPP4B) has a role of this antagonism effect by catalyzing the dephosphorylation of PIP2[16, 17].

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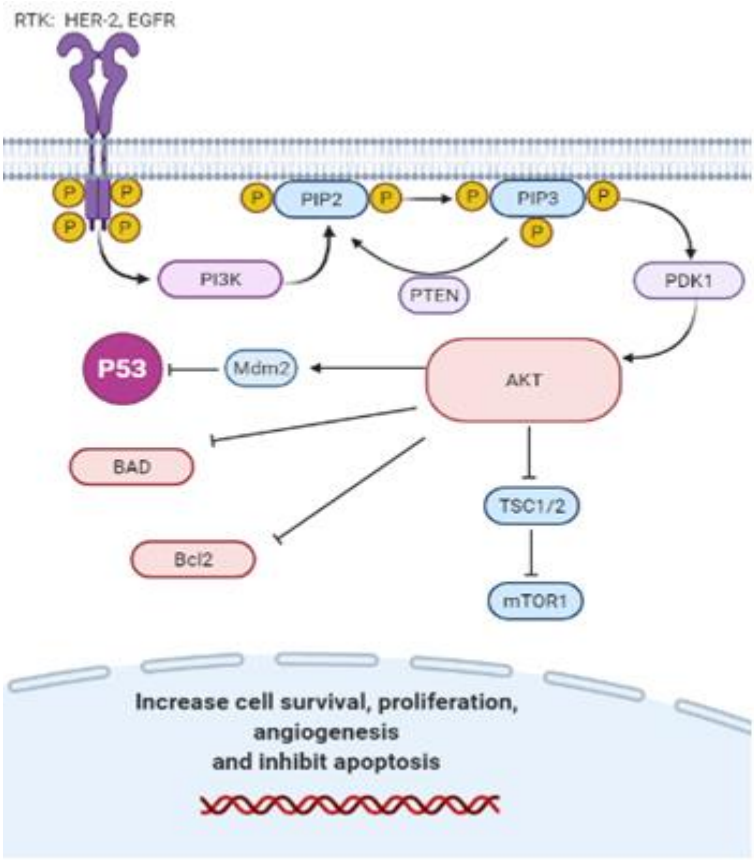

Figure 1: PI3K/AKT/mTOR pathway activation leads to enhanced cell survival, proliferation and diminished apoptosis

The frequency of PI3K/AKT/mTOR mutations in breast cancer:

Many of PI3K/AKT/mTOR pathway proteins are referred as oncogenes that activate the pathway signaling, others, tumor suppressor genes, can suppress the signaling through this pathway. In breast cancer, PI3K/AKT/mTOR activation encourages tumor progression and survival and mutations in the genes encoding several nodes of the PI3K/AKT/ mTOR signaling pathway have reported in ER+ breast cancer. These mutations indicates an significant role for PI3K signaling in the progression of ER+ breast cancer [16]. PIK3CA mutations, the most predominantly type of mutation in PI3K/AKT/mTOR signaling pathway, have been shown to trigger the PI3K pathway. 25% of breast cancer patients expressed PIK3CA mutations and it was shown that PI3KCA mutations were associated with the ER-positive and HER2-positive breast cancers. Also mutations in the PIK3CA gene which encodes the catalytic subunit of PI3K may hyperactivate the PI3K pathway (commonly found in luminal breast cancer and HER2-positive breast cancer) [18].

1.2. RAS/RAF/MEK/ERK signaling pathway:

Signaling cascade involves the association between multiple growth factors and their specific receptors. Usually, growth factors bound to and stimulate transmembrane domain of the RTK family and trigger a cascade of signal transduction that is transduced through multiple cytosolic messengers followed by signal transduction to the nucleus and activation of the transcription/translation of effector genes.RAS GTPases is the cytosolic mediators that trigger phosphorylation of the MAPK pathway. RAS GTPase encompass over 150 small G-proteins as H-RAS, K-RAS, N-RAS and others [19]. Guanine nucleotide exchange factors promote the activation of RAS to RAS-GTP via modulation of GDP/GTP cycling. While the inactivation is stimulated by GTPase-activating proteins that catalyze GTP hydrolysis into Ras-GDP which is the inactive form. The inactive RAS-GDP is present in normal quiescent cells where extracellular stimuli triggers the formation of Ras-GTP, the active form of RAS. This active RAS can bind to a numerous downstream mediators, of which the Raf kinases are the best recognized [19].

Activated RAS GTP interacts with and activates RAF, the downstream component of the pathway and phosphorylated RAF can subsequently activate MEK isoforms 1 & 2. MEK1 and MEK2 are considered attractive therapeutic targets as ERK can only be activated by them. The subsequent downstream effector of this pathway is

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ERK 1/2 that when activated detaches from cytoplasmic anchoring proteins, and translocates to the nucleus where they can impact the transcription of multiple genes via activating multiple transcriptional factors including Elk-1, CREB, Fos and globin transcription factor 1 (Gata-1). Those transcription factors can interact with promoters of numerous genes, including growth factor and cytokine genes as represented in (figure 2). These transcriptional modifications impact cell proliferation, survival, angiogenesis and abolish cellular apoptosis leading to cancer[20].

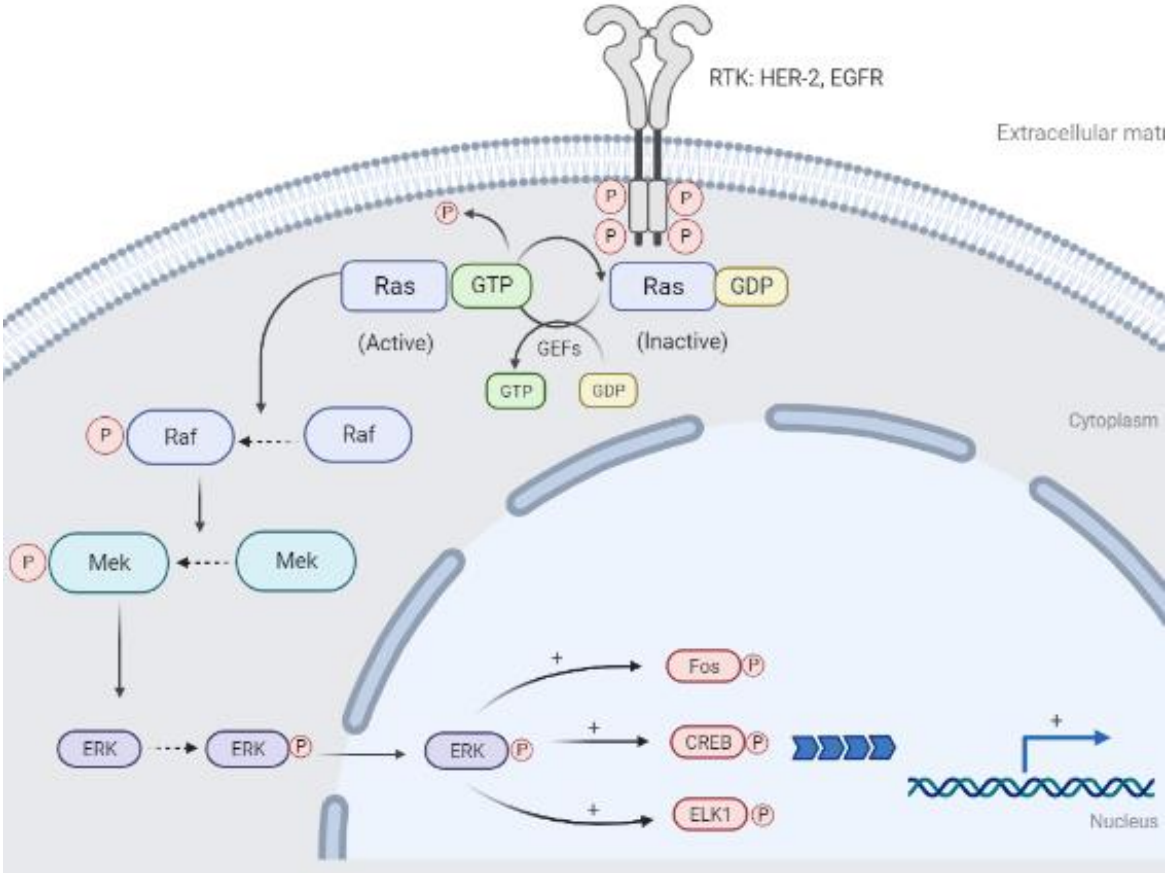

Figure 2: Activation of RAS/RAF/MEK/ERK following ligand recognition.

RAS/RAF/MEK/ERK pathway directing breast cancer:

Several actions of the RAS/RAF/MEK/ERK signaling pathway on apoptosis can be exerted via ERK phosphorylation of many proapoptotic proteins such as Bad, Bcl-2, CREB, Bim, Foxo, and Caspase-9. The Mcl-1 mRNAs translation that is implicated in apoptosis is also regulated by ERK activation. Regulation of apoptosis is seriously involved in breast cancer and inhibiting the main components in the apoptotic cascades is an attractive target of the pathway inhibitors [21].Mutations or amplifications of upstream growth factors receptors can also trigger the RAS/RAF/MEK/ERK signaling pathway. RTKs (e.g., EGFR and HER2) overexpression or mutations can cause hyperactivation of Ras, which in turn, triggers the RAS/RAF/MEK/ERK cascades. Overexpression and constitutive activation of EGFR in cancers is often correlated to poor prognosis. It has been suggested that prior to the diagnosis of breast cancer, EGFR levels may be elevated in the blood within 17 months [22].

HER2 activation results in transient stimulation of Ras, that triggers numerous downstream mediators activating cascade of signaling which modulate cell survival, proliferation, and differentiation. Furthermore, a truncated short EGF receptor has been observed in cases of breast cancer. This truncated protein loses 267 amino acids in extracellular domain resulting in a constitutively activate protein [21].Another link between Ras and EGFR is accomplished by the enhanced expression of EGFR ligands via Ras signaling. The pattern of activation of this signaling cascade in breast tumors proposes that it is not only critical in oncogenesis but also an attractive therapeutic target. The substantial experimental and genetic researches suggest strong rational that suppressing the RAS/RAF/MEK/ERK as well as EGFR pathways could afford effective anticancer therapeutic agents for breast cancer. Many inhibitors have been developed which could target diverse effectors of ERK signaling, some of them have already approved as antineoplastic agents [23].

PI3K/AKT/mTOR and Ras/Raf/MEK/ERK interplay:

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The cross interaction between MAPK and PI3K pathways is complicated and incompletely understood. It has been documented that, there is a substantial level of cross-interaction between kinases of both pathways in both physiological and pathological conditions. Studies revealed that, suppression of one pathway triggers the other one, and vice versa, so confirming that the signal is transduced downstream. Studies are now focusing on the multiple points of junction between the MAPK and PI3K pathways and the exact mechanism of cross talk are beginning to emerge[24]. MAPK pathway can regulate mTOR signaling by inactivating the TSC1/TSC2 heterodimer via ERK. Definitely, phosphorylation of ERK results in TSC1/ TSC2 secession and deteriorates the ability of TSC2 to suppress mTOR signaling. Moreover, ERK phosphorylates RSK1 that can interact with and phosphorylates TSC2 resulting in an enhanced mTOR signaling. Furthermore, it has been revealed that RSK & ERK can phosphorylates and activate another essential protein called RAPTOR that is mTORC1 complex scaffolding protein. Furthermore, the MAPK and the PI3K pathways intersect with the B cell leukemia 2 homology domain 3 (BH3)family of proteins, that modulate cellular apoptosis[25].

Augmented activity of the PI3K/AKT/mTOR pathway was observed following RAS/RAF/MEK/ERK pathway inhibition this might probably be because of loss of feedback suppression. Not only significantly amplified pAKT levels but also diminished pERK was observed in a preclinical study following MEK suppression, proposing the presence of an active feedback loop. Similarly, augmented activity in the MAPK/ERK pathway is resulted from blocking the PI3K pathway. Robust increase in pERK levels was reported in advanced solid tumor patients treated with mTOR inhibitor, suggesting stimulation of the MAPK pathway by a S6K-PI3K feedback loop [26]. This molecular course in breast cancer cells is further intricated by supplementary cross-talk of PI3K and ERK with another signaling pathways, such as ER signaling cascades. Using multiple kinase inhibitors to dually inhibit PI3K and MEK/ERK pathways results in higher growth attenuation than single pathway attenuation in various tumors.Moreover, single agent MEK inhibitor did not sufficiently inhibit basal-like breast cancer cells, while dual PI3K and MEK inhibitors leads to highly augmented growth suppression [27].

Conclusions:

In respect to the imperative roles of ERs signaling in diverse cellular processes and development, it is obvious that the mal-regulation of ER is implicated in tumorigenesis in breast cancer particularly in ER+ breast cancer. In addition to the typical hormone dependent signaling pathways, the pathways elucidated in this appraisal are shown to modulate various cellular functions that are imperative in the progression and onset of breast cancer. The study of EGFR family and its regulated pathways (RAS/RAF/MEK/ERK and PI3K/AKT/mTOR) represent a spectacular era of tumor research to enhance our consideration of molecular deviations that arise during tumor progression. Manipulating the critical role of these pathways in cellular survival and proliferation by modulation the nuclear translocation and transcriptional activity, may have a potential in the evolution of successful breast cancer therapy. However, the chief hurdles in effective breast cancer management embrace concomitant abnormalities and crosstalk between various intracellular signaling pathways in cancer cells which in conclusion culminate into the evolution of drug resistance and hence treatment failure. Therefore, several objectives need to be directed for superior clinical benefits and to reduce the evolution of drug resistance. Combined therapy including use of endocrine therapy along with inhibitors of numerous effectors of RAS/RAF/MEK/ERK and PI3K/AKT may be of tremendous benefits. This rationale provides tremendous potential for more efficient development of targeted therapy for breast cancer patients.

Declarations

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