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The Effect of Estrogen and Various Signalling Pathways in Breast Cancer Cells.

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ABSTRACT

Estrogens stimulate the proliferation and metastatic potential of breast cancer from its early stage. Its production and Estrogen Receptor action are key therapeutic targets leading to resistance against breast cancer. Based on a recent study, annually 1,44,000 new cases of breast cancer are reported in India.It has now become the most common female cancer in urban India. Cancer antigens are used as biomarkers for cancer diagnosis. It can be found in cancer cells, urine, blood or other body fluid that are expressed due to cancer cell growth. The two fundamental signalling pathways appear to be focused towards the breast cancer are the PI3K/AKT pathway and the JUN/MAPK pathway. The risk of breast cancer is not the same for all women in a given age group.However, research shows that factors such as: genetic alterations, lifestyle preferences etc increase the chances of breast cancer.

Keywords: Breast cancer - Estrogen– Estrogen Receptor- Biomarker- Signalling Pathway

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INTRODUCTION

Breast cancer is considered to play a major role in the cause of its cancer- related morbidity and mortality among women across the globe. Breast cancers emerge from the epithelial cells of the normal mammary gland [1]. Oestrogen is the primary sex hormone and is responsible for the regulation and development of the female reproductive system and secondary sex characteristics [2]. Estrogens play a major role in promoting the proliferation of both normal and neoplastic breast epithelium. Breast cancer is a malignancy whose dependence on the function of ovaries through the regression of both advanced cancer and metastatic disease induced by oophorectomy in women before menopause [3]. Prolonged lifetime exposure to estrogen is associated with elevated breast cancer risk in women says epidemiological evidence [4]. The effects of estrogens are exerted in the endometrium by means of two main classical estrogen receptor (ER) isoforms, ERa and ERb, and perhaps via G-protein-coupled estrogen receptor [5, 6]. Estrogen receptor (ER)-dependent and ER- independent are the two mechanisms by which estrogen exert their carcinogenic effects [6].

TYPES OF BREAST CANCER

Breast cancer can begin in many areas of the human breast – the ducts, the lobules, or in some cases, the tissue in between [7]. Breast cancer is if two broad categories: non-invasive and invasive.Non-invasive (in situ) breast cancers are cancerous cells stay in a specific area of the breast, without spreading to surrounding tissue, lobules or ducts.Invasive (infiltrating) breast cancers are cancerous cells break through normal breast tissue barriers and spread to other parts of the body through the bloodstream and lymph nodes[8].

- Endocrine positive- receptor (estrogen or progesterone receptors)
- HER2positive
- Triple positive which is positive for estrogen receptors, progesterone receptors, and HER2
- Triple negative: It is not positive for estrogen receptors, progesterone receptors, and HER2 [9].

HER2 is a protein that appears on the surface of some breast malignant cells. It might likewise be called HER2/neu or ErbB2. The imperative part of the pathway for cell survival and development is HER2 protein. A Ki-67 test is a typical approach to proliferation rate. At the point when cells are developing and multiplying, they make proteins called proliferation antigens [10].A cancer is estrogen-receptor-positive (or ER+) if it has receptors for estrogen. This suggests that the cancer cells, like normal breast cells, may receive signals from estrogen that could promote their growth. The cancer is progesterone-receptor-positive (PR+) if it has progesterone receptors. Once more, this implies that the cancer cells may receive signals from progesterone that could promote their growth. Two out of every three breast malignancies test positive for hormone receptors. On the off chance that the growth is hormone-receptor-negative (no receptors are available), then hormonal treatment is probably not going to work.[11].

DIAGNOSIS OF BREAST CANCER

- **BREAST EXAM:** Doctor will check both the breast and lymphnodes in the armpit, feeling for any protuberances or different anomalies.
- **MAMMOGRAM:** A mammogram is an X-ray of the breast. Mammograms are usually used to screen for breast cancer. In the event that a variation from the norm is distinguished on a screening mammogram, your specialist may recommend a diagnostic mammogram to further evaluate the breast cancer.
- **BREAST ULTRASOUND:** Ultrasound utilizes sound waves to deliver pictures of structures profound inside the body. Ultrasound might be utilized to figure out if another breast lump is a strong mass or a liquid filled pimple.
- **REMOVING A SPECIMEN OF BREAST CELLS FOR TESTING – BIOPSY:** A biopsy is the main conclusive approach for making a determination of breast malignancy. Biopsy tests are sent to a lab for investigation where specialists figure out if the cells are carcinogenic. A biopsy test is likewise dissected to decide the sort of cells required in the breast malignancy, the forcefulness of the disease, and whether the growth cells have hormone receptors or different receptors that may impact your treatment alternatives.

- **BREAST MAGNETIC RESONANCE IMAGING (MRI):** An MRI machine utilizes a magnet and radio waves to make photos of the interior of your breast.Prior to a breast MRI, you get an infusion of dye [12].

BIOMARKERS FOR METASTATIC BREAST CANCER:

Cancer antigens are used as biomarkers for cancer diagnosis. It can be found in cancer cells, urine, blood or other body fluid that are expressed due to cancer cell growth. Biomarkers in breast cancer are breast CA15-3, CA125, CA27.29, CEABRCA1, BRCA2, MUC-1, CEA, NY-BR-1, ING-1. These biomarkers include DNA modification, RNA, proteins, hormones, a molecule of the immune system and many other related molecules. Thus, various antigens can be identified and used as biomarkers for cancer identification and diagnosis [13].Estrogen receptor (ER) and progesterone receptor (PR): Examples of hormonal therapy used for metastatic breast cancer include the following:

- Aromatase inhibitors, which include anastrozole, letrozole, and exemestane
- Fulvestrant
- Tamoxifen

→ **Human epidermal growth factor receptor 2 (HER2)**

HER2 is a protein found in all breast cells, yet at different levels. At the point when a breast cell has an unusually abnormal state of HER2, it can drive breast cancer development and growth.Testing for HER2 helps specialists know whether a malignancy can be treated with anti-HER2 medications. These are the treatments that can prevent HER2 from helping tumor cells develop and spread. Anti-HER2 treatments include the following:

- Trastuzumab (Herceptin)
- Pertuzumab (Perjeta)
- Lapatinib (Tykerb)

→ **Cancer antigen 15-3, carcinoembryonic antigen.**

These biomarkers might be found in the blood of individuals with breast cancer.In any case, unusual levels of these biomarkers may likewise be an indication of another condition that is not a tumour [14].

ESTROGEN SYNTHESIS AND METABOLISM:

ESTROGEN SYNTHESIS:

In premenopausal women, estrogens are produced primarily in the ovaries, corpus luteum, and placenta, in spite of the fact that a little however a huge measure of estrogens can likewise be created by non-gonad organs, for example, the liver, heart, skin, and brain. There are three noteworthy types of physiological estrogens in females: estrone (E1), estradiol (E2, or 17β-estradiol), and estriol (E3). Every type of the estrogens presents diverse item conveyed from cholesterol by arrangement responses all through estrogen biosynthesis. E2 is the significant item from the entire biosynthesis prepare and is the most intense estrogen amid the premenopausal period in a lady's life, though E1 assumes a bigger part after menopause when it is orchestrated in fat tissue from adrenal dehydroepiandrosterone. E3 is the minimum powerful estrogen and is shaped from E1 through 16α-hydroxylation, assumes a bigger part amid pregnancy when it is created in vast amounts by the placenta. Estrogen deactivation, for example, a level of E2, can be directed by estrogens digestion systems, including change from E2 to a less-dynamic frame, for example, E1 or E3[15], and development of E2 sulfation by estrogen sulfotransferase to shape 17beta-estra-1,3,5-trien-3,17-diol 3-sulfate, which is no longer collaborating with estrogen receptors[16]. Moreover, examines demonstratedthat insufficiency of lipocalin 2, a novel fat determined cytokine, can likewise forbid E2 union by down direction of aromatase in fat tissue of female mice[17]. In this way, the proportion of coursing estrogens may serve as a characteristic of a dynamic digestion system: the harmony between estrogen combination and deactivation. The most widely used mechanisms for controlling estrogen synthesis in the body is regulation of the enzyme aromatase [18,19].

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ESTROGEN PRODUCTION IN THE OVARY:

- Estradiol is formed from the ovary, from the change of testosterone into estradiol by the chemical cytochrome P450 aromatase. This happens in granulosa cells.
- However, granulosa cells do not have the enzyme 17a-hydroxylase/ lyase, and in this manner, it cannot change over progesterone into androgens [20].

SOURCE OF ESTROGEN

Estrogen is discharged from the theca internal and granulosa cells of graffian follicle of an ovary. Interstitial cells, adrenal cortex, testis and placenta also secrete trace amount ofestrogen.

BIOSYNTHESIS OF ESTROGEN

Estrogens are a group of hormones synthesized in an assortment of tissues. 1 7p-estradiol (E2) is the essential estrogen of ovarian hormone. Cholesterol is the precursor of estrogenbiosynthesis. Cholesterol is metabolized to pregnenolone by P450 cleaved enzyme. Pregnenolone is then changed over to progesterone in the presence of catalysts 3β-OHSD and Δ5,4 isomerase. Inside the ovarian follicle, testosterone is formed either from progesterone or from pregnenolone like testosterone biosynthesis. Estrogens are framed by aromatization of androgens in an unpredictable procedure that involves 3 hydroxylation steps each of which required O2 and NADPH. The aromatase catalyst complex is thought to include a P450 mixed function oxidase. 1 7β-estradiol (E2) is principally formed from testosterone. Estradiol is promptly oxidized to estrone (E1) in the liver; estrone might be further hydrated to estriol[21].

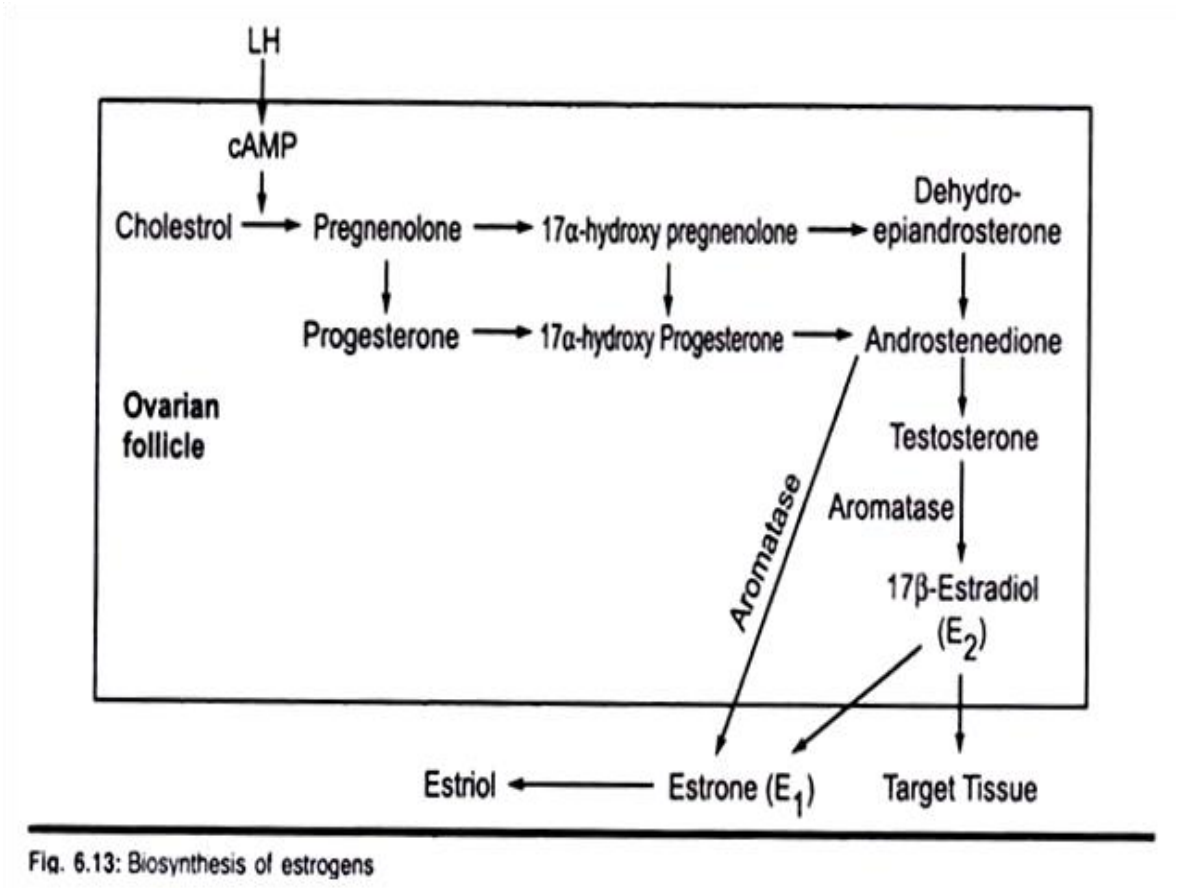

Fig. 6.13: Biosynthesis of estrogens

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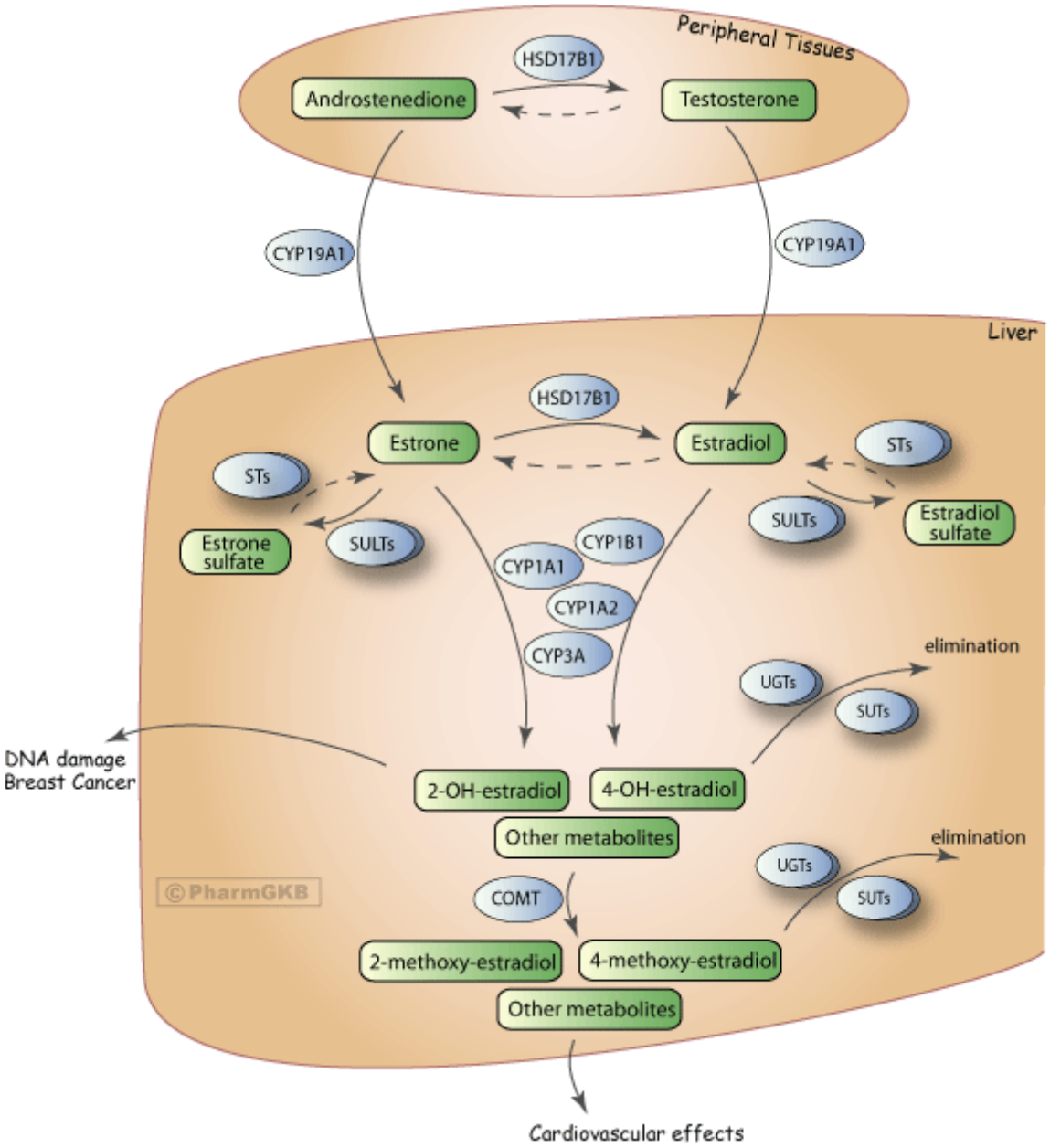
ESTROGEN METABOLISM PATHWAY:



DESCRIPTION

The liver is a site for biosynthesis of estrogens however it is likewise the fundamental site for further biotransformation of them. Once the estrogens are synthesized by aromatase in peripheral tissues including the liver, they will be discharged to the circulation. Estrone is in equilibrium with estradiol and 17-b-hydroxysteroid dehydrogenase (17bHSD) in this regard. The estrogens are taken up by the liver where they will be bio changed further into various metabolites. The major oxidative courses of estrone and estradiol are 2- and 4-hydroxylation by cytochrome P450 (CYP) 2B1, 1A and 3A. Other minor oxidative pathways are likewise recognized. The 2-and 4-hydroxy subsidiaries (and different metabolites) will be further changed over to 2-and 4-methoxy metabolites by catechol-O-methyltransferase (COMT). While the hydroxylated metabolites seem to bring about DNA harm and add to the tumorigenic impact of estrogen, the methoxy-subordinates seem to show helpful cardiovascular impacts. Estrone and estradiol and their metabolites experience sulfation by sulfotransferases (SULTs) and glucuronidation by glucuronyltransferases (UGTs). Estrone and estradiolsulfates could be deconjugated by sulfatases (STs) [22].

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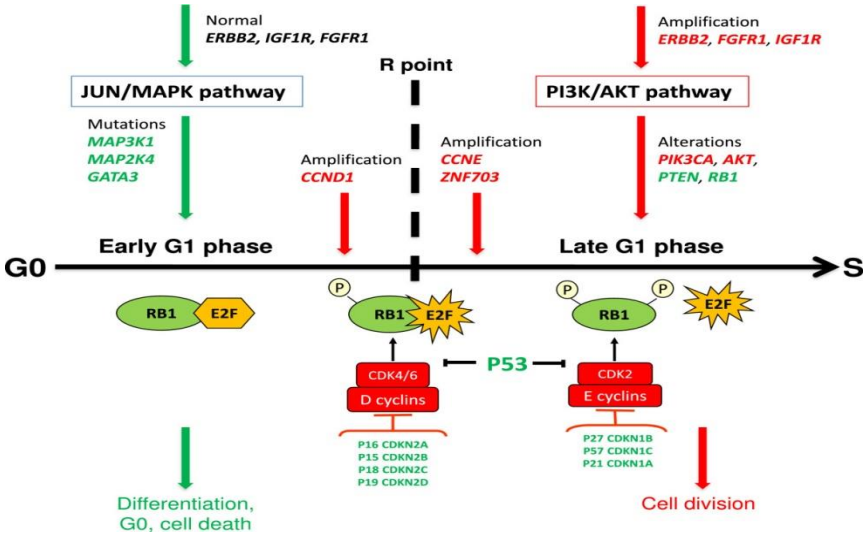
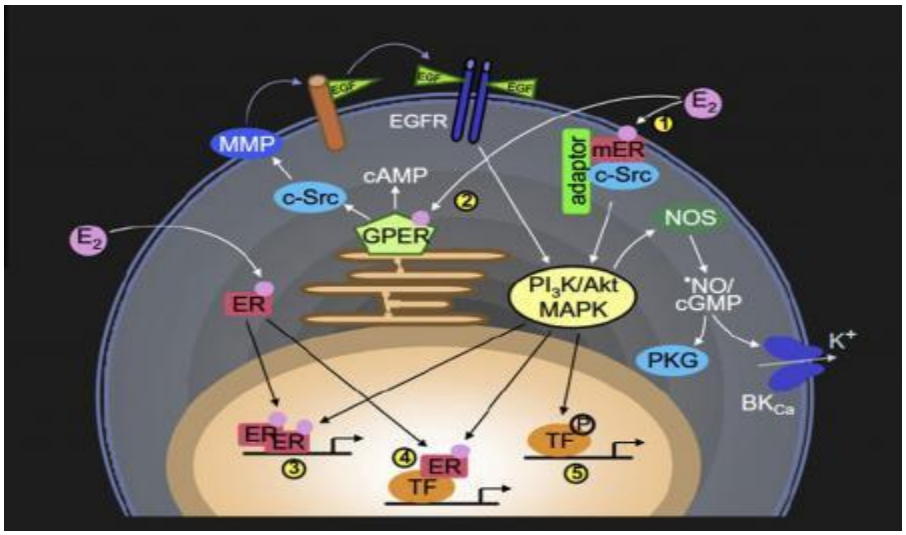
SIGNALLING PATHWAY



Two fundamental pathways appear to be focused on, the PI3K/AKT pathway and the JUN/MAPK pathway. Changes in segments of the PI3K/AKT pathway (PIK3CA, PIK3R1, AKTs, PTEN, INPP4B) are totally unrelated yet strikingly, intensification and upregulation of qualities encoding receptor-sort tyrosine kinases (RTKs) (IGF1R, EGFR, ERBB2) are likewise (all around) fundamentally unrelated with modifications of the PI3K/AKT pathway. This recommends the essential part of RTK enhancement or transformation is to actuate the PI3K/AKT pathway. In this way, in the typical mammary epithelium these RTKs are stifled or communicated at a low level and their flagging is essentially arranged toward the JUN/MAPK pathway, while when upregulated in tumour cells they invigorate the PI3K/AKT pathway. To get this measurement impact could be the explanation behind the enhancement of ERBB2 and FGFR1 genes, in spite of the fact that there could be different reasons [23]. It is realized that the PI3K/AKT pathway is initiated in tumour with changed EGFR or overexpression of ERBB2 and determines the response to ERBB targeted inhibitors [24]. Inside the JUN/MAPK pathway changes of the parts are additionally fundamentally unrelated. Segments of the JUN/MAPK pathway are inactivated by cancellations and transformations, for example, MAP2K4 and MAP3K1, or by enhancements, for example, PAK1. Above all, changes prompting to the actuation of the PI3K/AKT pathway and those promoting to the inactivation of the JUN/MAPK pathway are totally unrelated[25]. At long last, not just changes and genomic improvements influence qualities encoding segments of the two pathways yet inverse adjustments in expression examples of these qualities could likewise take an interest to their switch in breast disease [26].

ESTROGEN RECEPTOR (ER)-ACTIVATED PATHWAYS INVOLVED IN RAPID ESTROGEN SIGNALLING:



Natural (endogenous) 17b-estradiol (E2) activates membrane subpopulations of ERa and ERb (mER) that interact with adaptor proteins (adaptor) and signalling molecules such as c-Src (1), thereby changing the downstream ER-induced signalling through PI3K/Akt and MAPK pathways. E2 likewise binds to G protein-coupled estrogen receptor GPER, which is primarily present on the endoplasmic reticulum (2). GPER functions through activation of downstream effectors like adenylate cyclase (resulting in cAMP production), and c-Src; Thereby c-Src, in turn, activates matrix metalloproteinases (MMP), which cleave proheparin bound epidermal growth factor receptors (EGFR). The EGFR transactivation causes several intracellular responses, including activation of MAPK and PI3K. When once activated, PI3K stimulates NO generation by NO synthase (NOS) in smooth muscle cells (nNOS) or vascular endothelial (eNOS). Therefore membrane permeable NO, results in vascular smooth muscle cell guanylate cyclase activation resulting in cGMP production. The NO/cGMP pathway mediates vasodilation and events associated with signaling, such as protein kinase G (PKG)-dependent regulation of myosin light chain phosphorylation, and activation of large-conductance calcium-activated potassium (BKCa) channels. With regard to genomic, non-rapid signaling E2 also regulates cellular gene expression either via binding of ER dimers in the promotor region of target genes (3), also by the interaction ofER with different classes of transcription factors (TF) (4), or by regulation of transcription factor phosphorylation (5), [27].

WHAT TYPE OF HORMONAL THERAPY IS USED FOR BREAST CANCER?

A few procedures have been created to treat hormone-touchy breast disease, including the accompanying:

Premenopausal ladies, estrogen levels in these ladies can be decreased by taking out or stifling ovarian capacity. Blocking ovarian capacity is called ovarian removal.

Ovarian removal should be possible surgically in an operation to evacuate the ovaries (called oophorectomy) or by treatment with radiation. This kind of ovarian removal is typically perpetual. On the other hand, ovarian capacity can be smothered briefly by treatment with medications called gonadotropin-discharging hormone (GnRH) agonists, which are otherwise called luteinizing hormone-discharging hormone (LH-RH) agonists. These medications meddle with signs from the pituitary organ that invigorate the ovaries to create estrogen. Cases of ovarian concealment sedate that have been endorsed by the U.S. Sustenance and Drug Administration (FDA) are goserelin (Zoladex®) and leuprolide (Lupron®).

• **Blocking estrogen production**: Drugs called aromatase inhibitors can be utilized to hinder the movement of a compound called aromatase, which the body uses to make estrogen in the ovaries and in different tissues. Aromatase inhibitors are utilized basically as a part of postmenopausal ladies in light of the fact that the ovaries in premenopausal ladies deliver an excessive amount of aromatase for the inhibitors to piece viably. In any case, these medications can be utilized as a part of premenopausal ladies in the event that they are given together with a medication that smothers ovarian capacity.

Cases of aromatase inhibitors affirmed by the FDA are anastrozole (Arimidex®) and letrozole (Femara®), both of which incidentally inactivate aromatase, and exemestane (Aromasin®), which for all time inactivates the protein.

• **Blocking estrogen's effects:** Several sorts of medications meddle with estrogen's capacity to empower the development of breast tumor cells:

1**. Selective estrogen receptor modulators (SERMs)** bind to estrogen receptors, keeping estrogen from authoritative. Cases of SERMs endorsed by the FDA are tamoxifen (Nolvadex®), raloxifene (Evista®), and toremifene (Fareston®). Tamoxifen has been utilized for over 30 years to treat hormone receptor-positive breast malignancy.

Since SERMs tie to estrogen receptors, they can possibly not just piece estrogen action (i.e., serve as estrogen opponents) additionally imitate estrogen impacts (i.e., serve as estrogen agonists). Most SERMs carry on as estrogen opponents in a few tissues and as estrogen agonists in different tissues. For instance, tamoxifen obstructs the impacts of estrogen in breast tissue yet acts like estrogen in the uterus and bone.

2. Other anti-estrogen medications, for example, fulvestrant (Faslodex®), work in a fairly unique manner to piece estrogen's belongings. Like SERMs, fulvestrant appends to the estrogen receptor and capacities as an estrogen rival. Be that as it may, not at all like SERMs, fulvestrant has no estrogen agonist impacts. It is an unadulterated antiestrogen. Furthermore, when fulvestrant ties to the estrogen receptor, the receptor is focused for obliteration [28].

Tamoxifen citrate, which inhibits the activity of estrogen on breast tissue, improves disease – free survival among women who have estrogen receptor-positive breast cancer and reduces the risk of breast cancer [29,30,31].Raloxifen hydrochloride is a selective estrogen receptor modulator. It is chemically distinct from tamoxifen and estradiol, that binds to estrogen receptors to competitively block estrogen- induced DNA transcription in the breast and endometrium [32,33]. The second generation SERM that has estrogenic effects on bone and lipid metabolism, and ant estrogenic effects on breast tissue is the Raloxifene [34].

Tamoxifen and raloxifene may be useful in preventive therapies for women who have an alarming risk of estrogen receptor–positive breast cancer and vertebral fractures. Unfortunately, bone density have limited value in identifying women most likely to have overall benefit from these drugs because women with low bone density have a high risk of fractures but however a low risk of breast cancer [35,36]. Tamoxifen can be utilized by both premenopausal and postmenopausal women's, however, raloxifene is endorsed for use in postmenopausal ladies. These medications are named specific estrogen receptor modulators (or SERMs) on the grounds that they square estrogen in a few tissues of the body, yet act like estrogen in others [37]. Administration of 2000 IU/day of Vitamin D3, and, when possible, very moderate exposure to sunlight, could raise serum 25(OH)D to 52 ng/ml, a level associated with reduction by half in rate of breast cancer [38]

CONCLUSION

Breast cancer is said to be the most prevalent form of cancer in women. The leading cause of breast cancer is due to the impaired levels of estrogen. Estrogen,the primary female sex hormone and also plays a role in the treatment of certain hormone-sensitive cancers like that of breast cancer.Breast cancer at an early age is associated with the mutations in the BRCA1 and BRCA2 genes. Two-thirds of all breast cancers are fuelled by excess or unbalanced estrogens. A healthy weight is the best defense against hormone imbalance that can fuel a breast cancer diagnosis.

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