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Clinical Pathology of Breast Cancer: Staging and Biomarkers

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ABSTRACT

Based on recent statistics, one in every eight women will be diagnosed with breast cancer in their life. Breast cancer is classified into a staging system that ranges from zero to four, which is determined by several factors. In the staging system, the clinical pathology taken and tested by the healthcare professional plays a key role in determining the gravity of the patient's case. Biomarkers provide insight into the carcinogenic cells in the person's body, such as the Estrogen receptor (ER), human epidermal growth factor (HER2), and the progesterone receptor which are discussed in this paper. Each of these biomarkers communicates a specific attribute about the tumor cells and signifies the severity and the extent of the breast cancer. Based on this research, it was proven that there is not a direct correlation, but a slight correlation between the biomarkers found in the cells with the relative stage the patient has. Studies have shown a link between cells testing HER2 positive with high-grade tumors, unfavorable prognosis, lymph node collusion, high mortality rates, and higher rates of recurrence in patients which signify a stage three or four cancer. Studies also suggested that patients who test ER positive, PR negative, and HER2 negative are more likely to have early-stage invasive breast cancer, which alludes to stage one or two clinically. A definite conclusion was not able to be obtained as more research must be conducted, however, general trends were discovered between stages and biomarkers.

Introduction

Every two minutes, a woman in the United States is diagnosed with breast cancer since 1 in 8 women will develop the disease. This cancer is one of the most common seen by oncologists around the world, along with being the most common cancer among women. This cancer occurs when cells in breast tissue have mutations resulting in abnormal cells, which spread faster than healthy cells, causing lumps that metastasize to the lymph nodes or different parts of the body. Breast cancer is primarily identified with a biopsy, which is a device that is assisted by X-ray or another imaging technology to remove a sample of tissue from the suspected area of cancer. From here, a specialized doctor analyzes the results and forms a conclusion on how prolonged the cancer is. Doctors classify the different gravity levels of cancer with a staging system with four stages, stage one being the earliest stage and stage four meaning that cancerous cells have spread to other body parts. Biomarker testing is often used in cancer to help create specific treatment plans as a test that is a way to locate proteins or genes, which are called tumor markers or biomarkers, that provide insight into the carcinogenic cells in the person's body and about the cancer of the individual as it is unique to everyone. Cancer gets difficult to treat further along as it progresses through the stages, therefore, it is vital to diagnose and come up with a treatment plan early in the process. This paper will discuss the possibility of utilizing biomarkers found in the body to draw a correlation between the stages of breast cancer solely based on biomarkers.

Risk Factors and Prevention

Breast cancer is quite aggressive and is very commonly found in individuals across the world. It is the most common cancer for women compared to all other types. The reason for this is that there are many risk factors for breast cancer, some of which are very prevalent in the population, such as age. With age, breast cancer becomes more easily developable, making it a major risk factor. Furthermore, genetic mutations also play a key role in developing this cancer. Specifically, the gene mutation of BRCA1 and BRCA2 has led to breast cancer and ovarian cancer. These two genes are vital in cancer suppression as they are considered tumor suppressor genes. These genes facilitate for the cells to work normally and keep cells from growing or dividing in irregular ways.

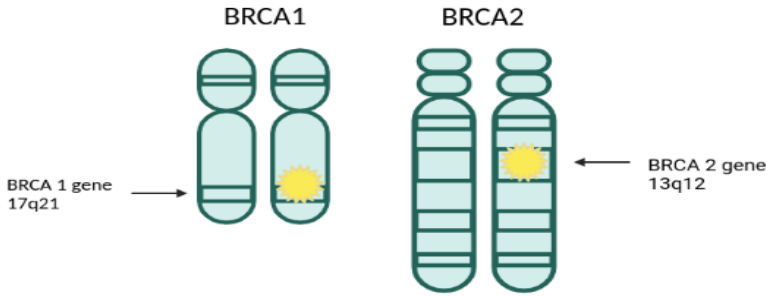

Figure 1. Mutation on the Chromosomes 17 and 13 of BRCA1 and BRCA2, Created in Bio Render, Copyrighted by Adithi Jonnagadla

When this gene is mutated, cells in the breast or ovaries are more likely to divide and change rapidly. Family history also greatly contributes to the risk of obtaining breast cancer, as mutations can be passed through the gene pool. However, many cases occur when the genes are mutated without any history of cancer in the family or in the individual. As a result of the prevalence of breast cancer in our society, many individuals seek prevention methods. Although there is a vast variety of suggested prevention techniques, the most recommended prevention technique is to increase physical activity. If an individual is at high risk for breast cancer, extensive measures will be taken. These measures can entail taking a variety of cancer-preventative medicines such as tamoxifen and raloxifene, to help prohibit the action of estrogen in breast tissue. Research for preventative measures and risk factors has increased leading to more public knowledge on reducing breast cancer.

Staging of Breast Cancer

The four stages play a vital role in the diagnosis and treatment of every cancer case. There is a range of stages of breast cancer from zero to four with zero being the earliest stage and four being the most severe one. In stage zero, the carcinoma is in situ, which means that abnormal cells appear like cancerous cells underneath a microscope. They are only found in one place of the formation and have not spread to any nearby tissue. As cancer progresses these cells may become cancerous and begin to diffuse to other nearby tissue that is healthy, as shown in the diagram below.

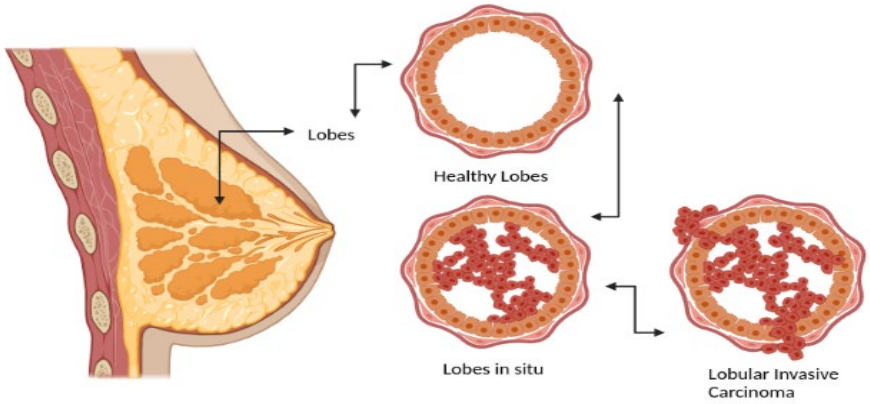

Figure 2. Carcinomas in Situ or Stage Zero, Created in Bio Render, Copyrighted by Adithi Jonnagadla

The consequential stages following stage zero measure the severity of the diffusion of cancer and the size of the tumor that is present in the body.

TNM Staging

Based on the specific type of cancer, the type of staging method used differs. For breast cancer specifically, the staging technique of the TNM system is used. This system is the one that is used in most cancers and is widely used as it is most hospitals and medical centers primary method for cancer reporting and staging. It follows clinical and pathological staging with the pathological staging being determined by extracting tissue surgically and clinical staging based on physical examinations, biopsies, and imaging tests if for any reason the patient is not to undergo surgery. For both staging systems, healthcare professionals raise seven different points of examination.

Extent or Size of Tumor (T)	Professionals look for the size of the tumor and if it has expanded into nearby areas.
Diffusion to Near Lymph Nodes (N)	Examined to see if cancer has grown into nearby lymph nodes and how many it had.
Metastasis to Distant Sites (M)	If cancer has spread or metastasized to distant organs, such as the lungs or liver.
Estrogen Receptor status (ER)	Cancer extracted is tested to see if it has a protein called an Estrogen Receptor.
Progesterone Receptor status (PR)	Cancer found is tested to see if cancer contains a protein called a Progesterone Receptor.
HER2 Status	Is assessed to see if the cells make too much of a protein called HER2.
Grade of Cancer (G)	Professionals observe if the cancerous cells appear as normal cells or look distinguishable and to what extent.

Figure 3. The Seven Points of TNM Staging

The letters “T, N, or M” provide information about the specifics of each of the points of the staging system. For T, it is often preceded by the number zero through four indicating the extent and size of the tumor and the extent of the spread around the body. Often, higher numbers next to T indicate that the tumor the patient has is on a larger scale and is widespread to tissues. T1 illustrates that the tumor is two centimeters across or less, T2 means that the tumor is within two centimeters and five centimeters, T3 indicates that the tumor is more than five centimeters across, and finally, T4 indicates that a tumor of any size is growing into the wall of the chest or into the skin, which is often referred to as inflammatory breast cancer.

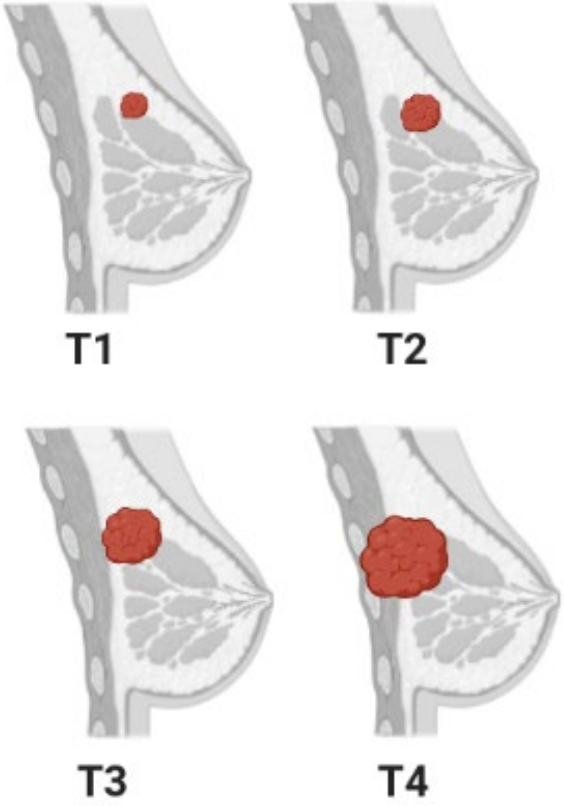

Figure 4. Four Levels of “T” in TNM Staging, Created in Bio Render, Copyrighted by Adithi Jonnagadla

The letter N is often preceded by the numbers zero through three. These numbers demonstrate if cancer has metastasized to lymph nodes that are located near the breasts and the extent that it has. If the areas of cancer are between 200 cells and 2000 cells, it is considered micrometastasis, which is only counted if there are not any large areas where cancer has spread. Areas that spread to lymph nodes that are larger than two millimeters or 2000 cells are considered an influence on the N stage. N0 demonstrates that there was not any cancer detected in the lymph nodes of the body, and N1 shows that cancer has spread to one to three axillary lymph nodes or on lymph nodes that are near the breast bone. Furthermore, N2 is classified when cancer is found in four to nine lymph nodes under the arm, or if cancer has swelled the mammary lymph nodes. Finally, N3 is characterized by having cancer in ten or more axillary lymph nodes and the mammary to collarbone lymph nodes. The letter M is followed by the number zero or one which indicates if cancer has metastasized to distant organs in the body, this can be in the kidneys, bones, liver, or lungs. M0 shows that there was no spread to these organs found on any imaging device or tests, and M1 illustrates cancer has been discovered in other distal organs, the most common being the bones, brain, and liver, but can include more. This was discovered on imaging devices or tests or concluded through a physical exam, with a biopsy proving that the cancer present is larger than 200 cells. The TNM staging system has proved complicated and each diagnosis is different for each individual, even if both patients are in the same stage.

Biological Markers of Breast Cancer

Another important piece of the breast cancer puzzle is the biological markers that are found in the body. Biomarkers, also known as tumor markers, are often used in cancer to help create specific treatment plans as a test that is a way to locate proteins or genes that provide insight into the carcinogenic cells in the patient. The two main types of biomarkers that are found often in breast cancer are biomarkers of exposure and biomarkers of disease. Biomarkers of exposure are often used in risk prediction and are looked at to infer the severity or the extent of the disease, on the other hand, biomarkers of disease are often used to diagnose and surveillance of disease progression. (Mayeux, 2004). Consequently, the specific types of biomarkers that are commonly discovered in breast cancer are tissue markers and genetic markers. Tissue biomarkers are biological macromolecules that are found in the tissue of the body and are used in pathology. Genetic markers are different types of molecules that can be DNA, RNA, protein or metabolomic profiles that are specific to the tumor. Testing can include genomic testing to look at the DNA sequence, DNA or RNA tests to look for gene fusions, or tests to measure RNA or protein levels (Kimmons, 2021). The three major biomarkers in breast cancer patients and in the TNM staging system are the Estrogen receptor (ER), Progesterone receptor (PR), and the human epidermal growth factor receptor (HER2). Biomarker testing is performed to establish hormone receptor status, which provides both prognostic and predictive value (Bernard et al, 2022).

Human Epidermal Growth Factor (HER2)

The biomarker, HER2, indicates the human epidermal growth factor of a certain individual. It is a transmembrane receptor tyrosine kinase located on chromosome 17q2 and is a member of the epidermal growth factor receptor (EGFR) family, which includes also EGFR (HER1), HER3, and HER4. These structurally related receptors are single chain transmembrane glycoproteins consisting of an extracellular ligand-binding ectodomain, a transmembrane domain, a short juxtamembrane section, a tyrosine kinase domain and a tyrosine-containing C-terminal tail. Binding of soluble ligands to the ectodomain of the receptor promotes homo- and heterodimer formation between receptors. Receptor dimerization is essential for activation of the intracellular tyrosine kinase domain and phosphorylation of the C-terminal tail. Phosphotyrosine residues then activate, either directly or through adaptor proteins, downstream components of signaling pathways (Moasser et al, 2011). This family of receptors play a vital role in a cell and help regulate cell proliferation, survival, migration, and differentiation and are classified as Tyrosine Kinases. They are also called ErbBs receptors and support development of eukaryotes. One of the main causes of cancer is losing function of one of the ErbBs receptors or a mutation occurring in the genome, however, this also leads to a vast majority of diseases in humans. In normal and healthy cells, the activation of this biomarker moderates cell signaling pathways which in turn help proctor the procedures of proliferation, motility, and survival (Viale, 2015). HER2 is found in almost all of our body cells with its main function being overseeing the relationship between different cells and helping them with their growth, division, repair, and survival. This is often considered as an oncogene, or a type of gene that helps contribute to the conversion of a normal cell to a cancerous cell in breast cancer. Specifically, this occurs when the HER2 gene is expressed at high levels that causes ligand- independent dimerization and leads to the activation of a cytoplasmic kinase domain. This activation leads to an unproctored awakening of the PI3K/AKT/mTOR and MAPK pathways which cause risk factors of tumor growth and progression such as cell proliferation, evading apoptosis, and angiogenesis (Viale, 2015).

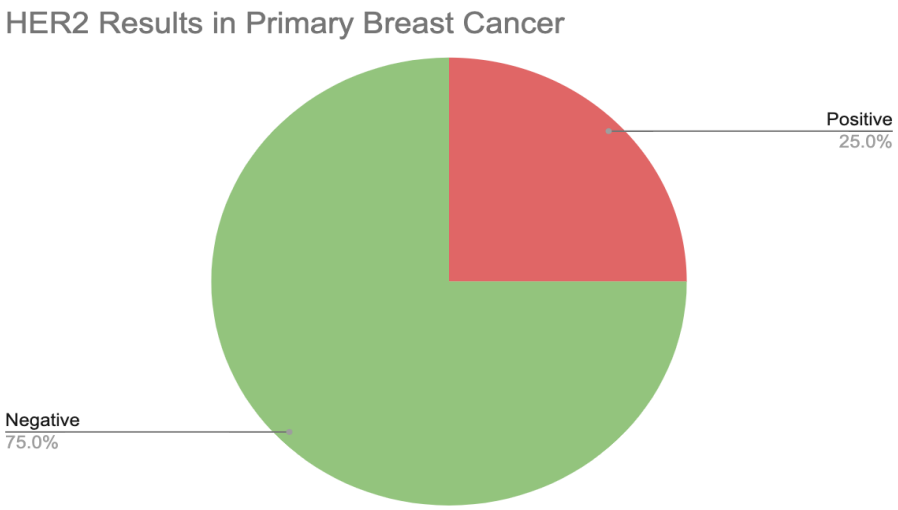

Figure 5. HER2 Test Results in Primary Breast Cancer, Created and Copyrighted by Adithi Jonnagadla

This is seen in some primary breast cancers which is due to the amplification of the gene that produces HER2 and dangerously facilitates tumor growth.

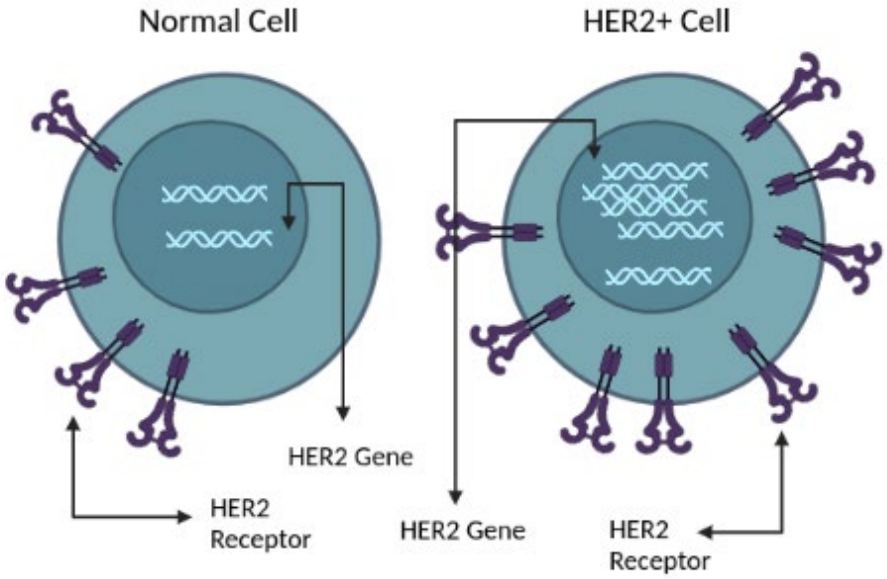

Figure 6. Normal Breast Cell Compared to a HER2+ Cell, Created in Bio Render, Copyrighted by Adithi Jonnagadla

HER2 is ranked on a scale from zero to three on a grading scale which helps determine the extent of the over-expressed gene.

HER2 Grade	Description	Interpretation
0	There is no reactivity present or reactivity is only shown in less than 10% of tumor cells.	HER2 Negative
1	Faint membranous reactivity is present in more than 10% of cells and the cells are immunoreactive only in part of the membrane.	HER2 Negative
2	Faint to moderate reactivity is shown in more than 10% of tumor cells.	Borderline Reactivity
3	Strong membranous reactivity is seen in more than 10% of cells.	HER2 Positive

Figure 7. Grading Scale of HER2

The HER2 gene is a vital biomarker that is utilized in the clinical pathology of breast cancer and is essential for learning about the severity of the cancer cells.

Oestrogen Receptor (ER)

The most powerful and common biomarker that is associated with breast cancer is the Oestrogen receptor (ER) with this marker expressed in 75% of tumors of breast cancer. The estrogen receptor (ER) is a ligand-activated transcription factor belonging to the nuclear transcription receptor superfamily and mediates the effects of the steroid hormone 17β-oestradiol in both males and females. They have a structure characteristic of members of the nuclear receptor superfamily, one of the largest protein families known to date with more than 70 currently recognized members. The members of this protein family are the receptors for testosterone, progesterone, corticoids, thyroid hormone, vitamins A and D3, and more. (Enmark. et al, 2001). There are two forms that exist of this biomarker, which are ER-a and ER-β. The former is associated in the clinical pathology of breast cancer although they are both transcription facts which subside the effects of oestrogen. They both combine to oestradiol in the same way, however, they show their individualism in the way they bind to other ligands and the byproducts produced from those actions (Payne. Et al, 2007). Steroid hormones like estrogen combine and activate ER, which then notifies its translocations to the nucleus. After it reaches its destination in the nucleus, the activated ER binds DNA and facilitates the transcription of genes from the DNA that are involved in proliferation, evasion of apoptosis, and angiogenesis (Viale, 2015).

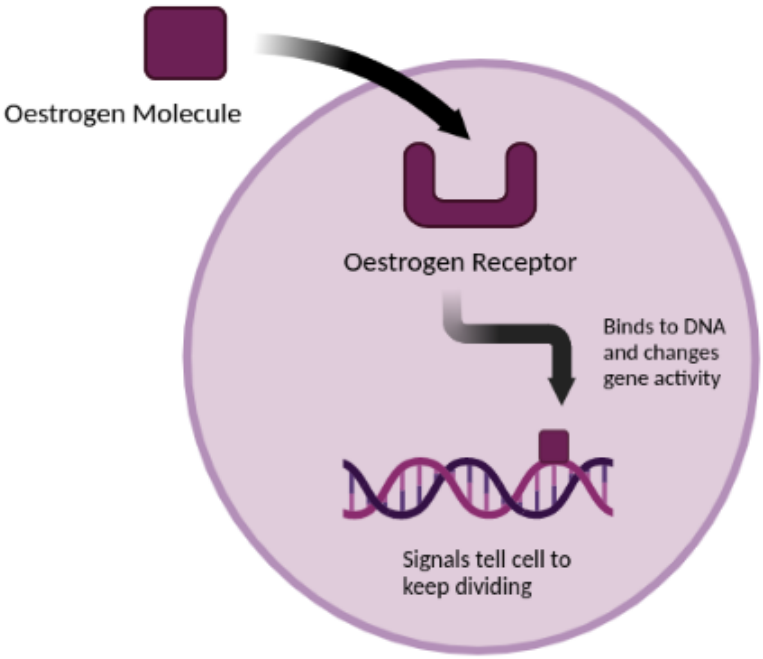

Figure 8. Oestrogen Receptor Activation, Created in Bio Render, Copyrighted by Adithi Jonnagadla

The reason that ER is considered to be one of the most powerful and vital biological molecules that are used in breast cancer is that it is the most informative for professionals. This biomarker holds the ability to differentiate between two distinct tumor subtypes, with the majority of luminal breast tumors expressing ER. ER is interconnected with the other biomarkers of breast cancer and helps shed light on the sub- cellular trends of breast cancer.

Progesterone Receptor (PR)

The final biological marker for breast cancer holds a similar relationship with ER, this biomarker is called the progesterone receptor (PR). This receptor is a member of the nuclear hormone receptor family of ligand-dependent transcription factors. Progesterone and oestrogen are both steroid hormones that combine and activate PR which then leads to the transcription of genes involved in several cellular processes, including proliferation, evasion of apoptosis, invasion, and angiogenesis (Viale, 2015).

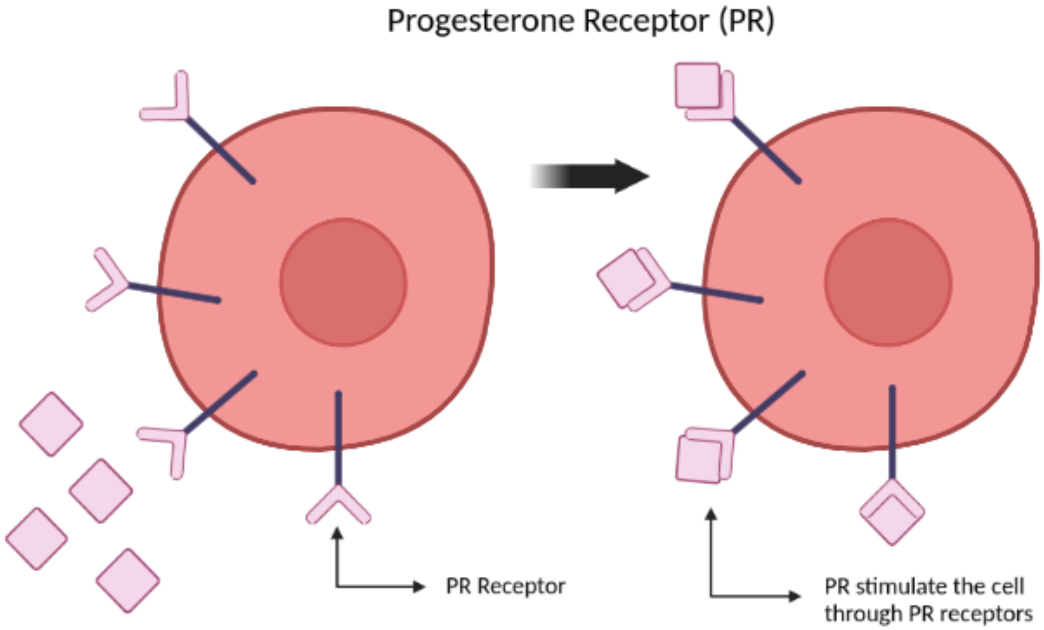

Figure 9. Progesterone Receptor Activation, Created in Bio Render, Copyrighted by Adithi Jonnagadla

This biomarker is expressed in two different forms, PR-α and PR-β that regulate different but overlapping subsets of target genes. PR is a gene that helps speed up the process of the ER and is highly dependent on oestrogen, hence these two biomarkers go hand in hand. Therefore, when tumor cells are tested, it is often seen that cells that are concluded as PR positive also are ER positive as PR is expressed in >50% of ER-positive breast tumors but rarely seen in ER-negative breast tumors. Consequently, the absence or presence of PR proves productive in defining the triple negative breast cancer subtype, which is when the cancer cells test negative for all three biomarkers, PR, ER, and HER2 which occurs in 15% of breast cancers. (Viale, 2015). The presence of PR is similarly determined to the presence of ER, with a biopsy taken of the cancerous cells in the body and then tested for the biomarker. The progesterone receptor is very commonly used in the diagnosis of breast cancer and goes hand in hand with ER which helps determine tumor severity.

Ki-67 Protein

The Ki-67 protein is also frequently used in order to stage and diagnose breast cancer as it is associated with cell proliferation. Among several methods for assessing cancer cell proliferation, Ki-67 assessment is probably the best known. A monoclonal antibody is used for IHC staining of the proliferation-associated nuclear protein Ki-67 in tumor cells to determine the percentage of Ki-67–positive cells among the total population of tumor cells in formalin-fixed paraffin-embedded sections obtained from a core-cut biopsy sample; this is the Ki-67 index (Lartigue, 2021). During the process of interphase in the cell cycle, this Ki-67 protein antigen can be exclusively detected within the nucleus. However, during the process of mitosis, the protein is relocated to the top of the chromosomes and is very active during cellular reproduction. Consequently, it is present and active during all phases of the cell cycle including G1, S, G2, and mitosis with it inactive in G0, the resting phase. These traits of the protein facilitate the conditions of a productive biomarker in order to determine the growth fraction of a given cell population (Scholze. et al, 2000). Ki-67 has been used to determine the rate of tumor

growth for many years, but there have been issues around the consistency and reproducibility of clinical scoring of Ki-67 levels (Vaughan, 2022). However, through research and biotechnological innovation, this problem has mostly subsided. This protein is most likely expressed when tumor cells divide and multiply, therefore, indicating an aggressive tumor growth. This type of growth is most commonly seen with high grade cancers and higher stages of breast cancer such as stages 3 and 4. Tumors are classified as having a high or low Ki-67 index based on a prespecified cutoff. Tumors with a high Ki-67 index have a larger number of proliferating cells and are therefore likely to grow more quickly (Lartigue, 2021).

Subgroups of Molecular Invasive Breast Cancer

The types of breast cancer are broken down into five different subgroups of molecular invasive breast cancer. The molecular subtype of an invasive breast cancer is based on the genes the cancer cells express, which control how the cells behave (DePolo, 2022).

Type	HER2	ER	PR	Ki-67 Protein
Luminal A	Negative	Positive	Positive	Low levels
Luminal B	Positive	Positive	Negative	High levels
Luminal B-like	Positive	Positive	Can be Negative or Positive	All levels
HER2 Enriched	Positive	Negative	Negative	N/A
Triple Negative or Basal-like	Negative	Negative	Negative	N/A

Figure 10. Molecular Subtype of Breast Cancer Correlation with Biomarkers

The type with the least severity is called luminal A. This type of breast cancer has low levels of the protein called Ki-67, shown in the table above. This protein facilitates how fast cancer cells grow in the body. For Luminal A cancers, tumors tend to grow slower and tend to be lower grade, thus, resulting in a good prognosis for the patient. In luminal B and in luminal B-like cancers, they both tend to grow quicker than luminal A and have a worse prognosis. The Ki-67 index was shown to discriminate between the luminal A and luminal B subtypes most effectively when a cutoff of 14% was used, since Ki- 67 acts as a productive distinguisher. Distinguishing between Luminal A and Luminal B cancer can guide decisions about the need for added chemotherapy. Because the cost of gene expression analysis has limited its adoption in clinical practice, protein expression levels of ER, progesterone receptor (PR), and HER2 can be used as surrogate markers for subtype classification.For ER-positive, HER2-negative breast cancers, Ki-67 has emerged as another important surrogate marker because luminal A tumors typically have a lower rate of proliferation than luminal B tumors (Lartigue, 2021). Furthermore, HER2 enriched cancers grow quicker than luminal cancers and have a worse prognosis, however, they can be treated with procedures that target the HER2 biomarker. Finally, for triple negative or basal-like cancers, these are considered more aggressive than both luminal A and luminal B cancers and are most commonly found in black women, younger women, and people who have a BRCA1 mutation in their chromosomes. There is a correlation between the biomarkers and the five subtypes as shown in the table and professionals often use these parameters to help come up with treatment plans and learn about the severity of the patient's cancer.

Conclusion

After looking at the research that has been conducted on the biomarkers of ER, PR, and HER2, we can draw some conclusions about how they fit into the staging process. As seen with the different sublevels of cancer, the biomarkers found in cancerous cells do correlate with the severity of the tumor. This is due to the fact that many studies have affirmed the link between cells testing HER2 positive with high grade tumors and an unfavorable prognosis. It is also shown that HER2 positivity is associated with lymph node collusion, high mortality rates and higher rates of recurrence in patients which signify a stage three or four cancer. Paralelly, studies suggest that patients who test ER positive, PR negative, and HER2 negative are more likely to have early stage invasive breast cancer, which correlates to a stage one or two clinically. Therefore, we can conclude that there is a slight correlation with the severity of the cancer with the biomarkers present, however there is no exact stage correlation. In order to determine the specific stages with the status of the biomarkers, more studies must be conducted to gather data from cancerous cells and be able to draw trends solely based on the biomarkers present. Researchers have expressed their limitations with the three main biomarkers discussed here, so to hopefully draw a more thorough conclusion, researchers should explore further less known biomarkers to signify specific clinical staging.

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