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A Hormonal and Cell Cycle Centered Approach to Breast Cancer

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ABSTRACT

In the year 2022 alone, almost 2 million new cancer cases have plagued just the United States, breast cancer being the most threatening for women (Łukasiewicz et al., 2021). It is important to advance our scientific understanding of cancer, to improve our surgical techniques, and to administer more efficient chemotherapy. While all of this is true, it is just as important to focus on prevention. To focus on the fundamental understandings of the cell cycle and hormones, and how they can play a major factor in preventing breast cancer. And to also focus on finding compounds like Indole 3 Carbinol which is present in our food, to teach us how certain environmental and lifestyle changes might keep us healthier for longer and prevent mutations in our cell cycles.

Introduction

Breast cancer is one of the among the most common cancers in women worldwide, both in developed and developing countries, and has the highest incidence in women over the age of 50. (Łukasiewicz et al., 2021)

The formation of breast cancer is preceded by the initiation, fixation, duplication, and promotion of a mutated breast cancer cell. Once a cell is mutated, it must undergo multiple rounds of cell division in the right conditions to become established and be able to develop into a cancer. During the cell cycle, the mutated cells must overcome the various mechanisms the body has in place to prevent the development of cancer such as apoptosis and damage recognition systems (Schafer, 1998).

To understand how cancer cells proliferate, the cell cycle must be understood. In the cell cycle there are key checkpoints and regulators to prevent mutations and cancerous cells from replicating. There are four stages of the cell cycle: G1, where the cell grows in size and starts cultivating the nutrients it needs to grow; the S phase, or synthesis phase, in which cell DNA begins to be replicated; and the G2 phase where the cell prepares for the duplication that takes place in the M, or mitosis phase (Schafer, 1998).

Within this process, there are special checkpoints and regulations to prevent mutations. First is the G1 checkpoint, at this point the cell is thoroughly checked for sufficient cell growth, nutrients, growth factors, and any DNA damage (Figure 1). After a cell passes through the G1 checkpoint, it is committed to the cell division process and forfeits its ability to regress into previous phases of the cycle. The next checkpoint is the G2 checkpoint which determines that DNA replication is completed correctly and that there are no mutations or damages in the cells. If there is a mutation present, the cell will be repaired in the G2 phase, but if the damage is beyond repair, apoptosis, or programmed cell death will be induced (Murray, 1994).

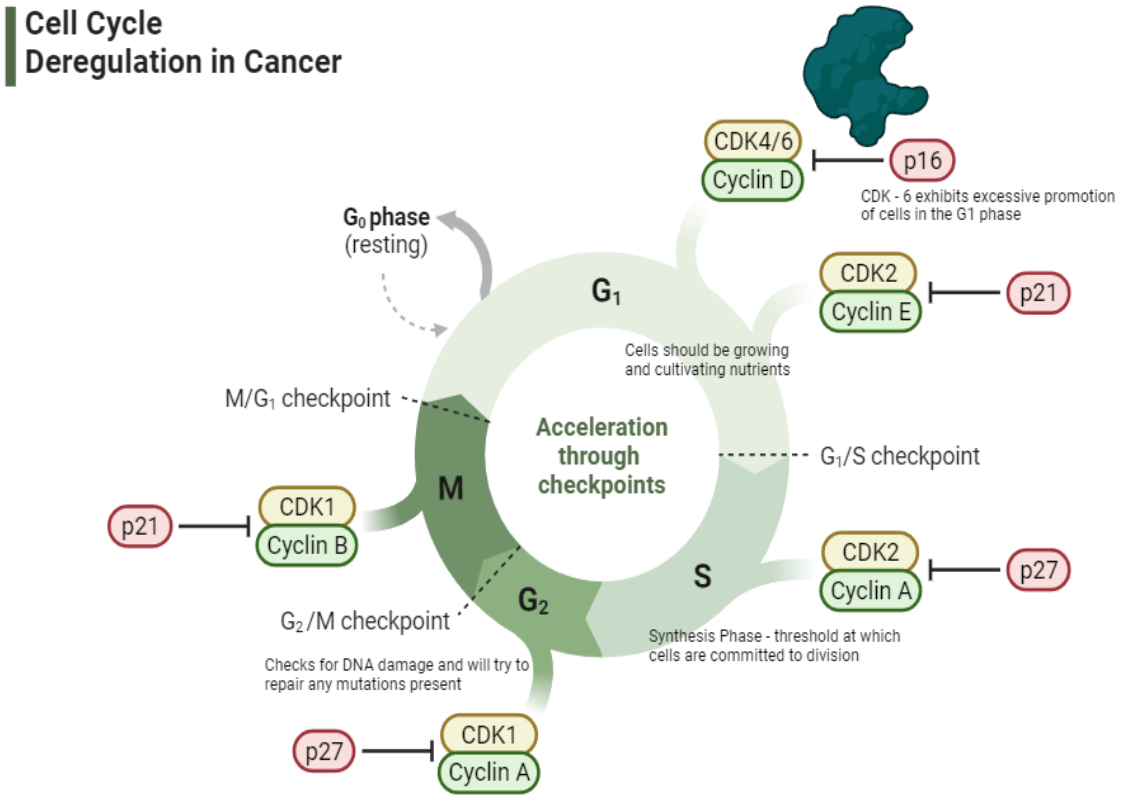

Figure 1. The cell cycle broken into the checkpoints and phases and the cyclins and CDKs labeled respectively. Created in Biorender

The reasons these checkpoints are successful is due to cell cycle regulators, like cyclins. Cyclins are proteins associated with the phases of the cell cycle and promote activity in their particular phase. For the cyclins to become active and be able to modify target proteins it must bind to a CDK (Cyclin Dependent Kinase) enzyme. When attached on a particular site, the phosphate acts like a switch that determines the level of activity in a target protein. However, in breast cancer, CDK-6 especially, which is most active in the proliferation of cells is unbalanced and is difficult to turn "off." This paper will touch on the hormonal and genetic factors that have this effect on the cell cycle and how natural treatments can target CDK-6 (Murray, 1994).

There are many risk factors that can reduce the body's ability to recognize and fix mutated cells therefore inhibiting the regular function of the cell cycle. In the case of all cancers, factors like old age or exposure to carcinogens decrease the capabilities of the cell cycle and how precisely and quickly it can recover. In breast cancer, there are many risk factors that make it the most common cancer for women worldwide.

60% of cancer in the breast starts out being hormone dependent. This means many risk factors associated with breast cancer will be associated with hormones, along with methods of therapy. However, hereditary mutations in breast cancer can exist, like BRCA1 and BRCA2 mutations which are carried by 0.25% of the population (BRCA: The breast cancer gene - BRCA mutations, 2022).

BRCA1 & BRCA2

BRCA1 and BRCA2 produce tumor suppressor genes. This means that normally, these genes are essential in combating cancer. That is why hereditary mutations of this gene are dangerous. When the BRCA1 or BRCA2 gene is mutated its function of DNA repair in preventing breast cancer goes away and individuals that inherit this gene are more likely to develop breast cancer. 55% of patients diagnosed with the BRCA1 mutation and 75% of patients with the BRCA2 mutation are likely to be diagnosed with breast cancer by or before the age of 70 (BRCA: The breast cancer gene - BRCA mutations, 2022).

BRCA1 and BRCA2 are important as tumor suppressors because they produce proteins like ATM and CHK2 that are hugely required in the cell cycle process as DNA repair and damage recognition proteins. In the G1 phase of the cell cycle, BRCA1 and BRCA2 assist in maintaining genome stability by repairing DNA damage, and when these cells progress into the S phase, where DNA replication occurs, their job is to ensure there are no mutations in the replication. When BRCA1 and BRCA2 are mutated, DNA repair function and cell damage recognition reduces, and the likelihood of chromosomal abnormalities and mutations leading to cancer is increased (BRCA: The breast cancer gene - BRCA mutations &, 2022).

Hormones and Breast Cancer

BRCA1 and BRCA2 are risk factors associated with non-hormonal breast cancer, but the majority of breast cancer is hormonal, and the incidence of breast cancer can vary based on stage of life and circumstance.

Studies have shown there is a relationship between reproductive history and the incidence of breast cancer which has to do with the main hormones involved in breast cancer, which include estrogen, progesterone, and growth hormones (Gompel, 2019). Women that go through full-term pregnancy before the age of 30 have a reported 50% decline in growth hormone, suggesting pregnancy can be protective against breast cancer (Gompel, 2019).

This is because of the fundamental link between growth hormone and estrogen. Growth hormone stimulates estrogen receptor expression. When early full-term pregnancy decreases growth hormone, there is a decrease in estrogen, meaning less promotion within the cell cycle in the G1 phase, which results in lower incidences of cancer. Estrogen is a hormone essential to the development of the female reproductive system, but when estrogen levels are high, cell growth and proliferation is increased, which increases the likelihood of mutations (Santen et al., 2022). Estrogen receptors on breast cancer cells, when bound with estrogen, activate cell division and increase the incidence of cancer (Łukasiewicz et al., 2021). So, when the expression of estrogen receptors decreases, there are lower incidences in cancer. That is why pregnancy can be protective. The initial offset of growth hormone is created because of differentiation, meaning cells are focused on lactation rather than growth.

In the past, scientist Dr. Jose Russo hypothesized that the differentiation of breast tissue stops the growth of breast cancer cells because, during pregnancy, the terminal end buds of the breast disappear (Russo et al., 2005). Terminal end buds are highly populated with rapidly dividing cells and were thought to be the location in the breast where cancer is initiated. Russo's claim was that pregnancy was protective because, during pregnancy, terminal end buds would disappear, so there could be no initiation of cancer. To test this, rats were treated with perphenazine to spike prolactin levels to induce full differentiation. However, when these pregnant rats were exposed to MNU, a direct-acting carcinogen, full differentiation did not stop them from getting cancer, indicating that pregnancy protection is attributed to a more complicated circulation of hormones, as mentioned earlier (Russo et al., 2005).

Within a woman's lifetime, estrogen levels are constantly changing, making it one of the biggest risk factors for breast cancer in women (Gompel, 2019). As mentioned, pregnancy can be protective due to a decrease in estrogen, but late full-term pregnancy or nonpregnancy has the opposite effect. This is because of the general rule that the more time women are exposed to hormones like estrogen, the more their likelihood of cancer increases. This can happen with early periods and late menopause because of how heavily estrogen is involved in these cycles (Santen et al., 2022). Estrogen can also be found in small amounts in fat cells, meaning obese women, especially after menopause, have a higher incidence of breast cancer.

Luminal and Triple Negative Breast Cancer

The function and activity of hormones within breast cancer can change the classification of cancer being dealt with and the types of treatments associated with a specific diagnosis.

Luminal breast cancer accounts for around 70% of breast cancer in the Western world and acts as an ER-positive tumor, meaning the breast cancer cell receptors use estrogen to allow themselves to multiply (Łukasiewicz et al., 2021). Luminal cancer comes in A and B subtypes characterized by proliferation-related pathways and luminal-regulated pathways. The difference occurs between these pathways because there are specific genes and proteins expressed more heavily in each pathway. Luminal A tumors typically have a lower expression of proliferation-related genes and a high expression of luminal-regulated genes (ESR1, FOXA1, PR). Luminal-regulated genes are involved in the normal function of luminal cells in the breast. In the hormonal sense, Luminal A is characterized as ER/PR positive, and HER-2 negative, meaning the breast cancer cells use more estrogen and progesterone to grow (Yersal, 2014). On the other hand, Luminal B tumors often have higher expression of proliferation-related genes (MKI67 and AURKA) and lower luminal-regulated genes, meaning they are more focused on division than function. They are ER-positive and are either HER-2 or PR-negative. Luminal B tumors are typically of higher grade and have a much worse prognosis (Yersal, 2014).

The poor prognosis of Luminal B brings up the question of estrogen's role. Both Luminal A and B are estrogen-positive, but Luminal B has mixed responses to hormone therapy and is more aggressive. The presence of estrogen ensures the presence of hormone receptors that can be altered with hormone therapy. However, Luminal B does not respond to hormone therapies like tamoxifen or aromatase inhibitors because of its higher levels of growth factor receptors like HER-2. Hormonal therapies work to block estrogen receptors, but HER-2 promotes cell division in G1 and can activate signaling pathways like, PI3K/AKT/mTOR, that bypass estrogen signaling and make Luminal B less responsive to hormone therapy (Yersal, 2014). The proliferation of cells also creates an opportunity for mutations to be formed which makes hormone therapy less effective. This is why, even when Luminal B is ER+, it is still difficult to treat with hormone therapy.

The prognosis of Luminal B breast cancer is similar to that of Basal-like breast cancer which accounts for up to 37% of cancers. This type of breast cancer has a very poor prognosis and aggressive clinical behavior usually metastasizing into the brain and lung region (Kim 2021). Tumors in the basal-like group exhibit high levels of CK5, CK 14, CK 17 and laminin, which are markers of basal cells (Yersal, 2014). However, they do not express ER, PR, or HER-2 which are the hormones usually correlated with breast cancer. Due to its failure to express these hormones, it is named triple-negative breast cancer (TNBC).

TNBC makes up to 80% of breast cancer related to BRCA1, which as established, is a hereditary mutation in tumor suppressor genes that is likely to lead to cancer. Conversely, up to 16% of TNBC happens in the BRCA1 or BRCA2 genes (Venkitaraman, 2002). This is why BRCA1 is amongst the most difficult risk factors when it comes to breast cancer. Not only can it be hereditary, but it also has high levels of correlation with the most aggressive subtypes of cancer. As mentioned, 60% of cancers are hormonal, but since TNBC does not express ER, PR, or HER-2, it can't be treated with hormonal therapy since it does not express the receptors that would be targeted in treatment. TNBC also tends to be more genetically unstable, so alternative treatments are even harder to create. This is because the instability of the genome can create new mutations and

adapt to the effects of the treatment. BRCA1 and BRCA2 often being a part of the TNBC subsect explain why it is so hard to treat (Piezzo et al., 2020).

Understanding Where Cancer Grows – Metastasis

Breast cancer is extremely complex, known by the number of subtypes it can have, but its effects throughout the body can also vary. Metastasis is when cancerous cells from a primary tumor travel through the blood or lymph systems and relocate to different organs or tissues of the body (Kim, 2021). In 1889, Stephen Paget observed that metastasis in organ distribution was intentional via data that had been gathered from 735 women with breast cancer. Paget suggested that the place of metastasis intentionally so the cancer could grow under the best conditions. A seed needs specific conditions and soil to germinate and flourish. Just like a seed, cancer cells need to be in a very specific microenvironment within the body to establish themselves and proliferate. For breast cancer cells, the most "nurturing" place in the body is the bone. Breast cancer is one of the most common carcinomas that develop bone metastasis, 67% going toward the bone. Within breast cancer's likelihood to spread to bone, Luminal A and B subtypes account for about 70% to 80% and TNBC has around 40% likelihood of bone metastasis (Tufail M et al., 2022). Studies suggest that the microenvironment that makes bone metastases so ideal are bone-derived chemokines, such as osteopontin, osteonectin, and stromal-derived factor-1 (SDF-1:CXCL12) that attract breast cancer cells to the bone. The activation of CXCR4, a receptor for CXCL12, which is expressed in breast cancer cells, stimulates cellular processes and paths that promote metastasis and breast cancer cell growth in the area (Tufail M et al., 2022). These processes include cell proliferation, cell survival, and migration, which are necessary for breast cancer cells to travel from the initial breast cancer site to the bone and establish bone metastases. So what are some ways to prevent cancer from spreading this far? To prevent resorting to invasive surgeries and debilitating chemotherapy treatments?

Indole 3 Carbinol: Diet and Breast Cancer

Diet has an integral contribution to how our bodies react and function, especially at a cellular level. Indole-3-Carbinol (I3C) naturally occurs in Brassica vegetables, including broccoli, cabbage, and Brussel sprouts, and has been proven to reduce the incidence of carcinogen-induced breast cancer. When I3C was administered to rats before exposure to the carcinogen, the incidence of breast cancer induced by 7,12-dimethyl-benz(a)anthracene or DMBA, a potent carcinogen, decreased by 70% to 90% (Cover et al.,1998)

I3C inhibits the growth of breast cancer cells in a dosage and time-dependent manner. When breast cancer cells are treated with I3C, they arrest at the G1 phase of the cell cycle and are prevented from multiplying. There is a mechanism behind this. Breast cancer cells arrest at the G1 phase because I3C reduces the production of CDK6 or Cyclin Dependent Kinase 6, which is a protein necessary in the cell cycle for proliferation and division, but is overly active in breast cancer (Piezzo et al., 2020). If breast cancer cells can get to the S phase with the help of promotion from CDK-6, it can lead to the formation of a tumor, and makes it more difficult to eradicate the cancer. The implementation of I3C in diet can thus help prevent the division and proliferation of MCF7 breast cancer cells by reducing CDK-6 activity. (Cover et al.,1998).

Additionally, I3C was shown to prevent ER-positive and ER-negative breast cancer cell subtypes, suggesting that it might be effective in treating multiple types of cancer. This was done with a triple negative breast cancer cell line MDA-MB-231, which lacks estrogen, progesterone and HER2 receptors. IC3's ability to reduce the incidence of cancer in MDA-MB-231, creates hope for finding new treatments for TNBC, which is far more aggressive and difficult to treat due to the lack of hormone receptors. Researchers have also experi-

mented with using IC3 with the antiestrogen drug tamoxifen. This combination yielded the most effective reduction in the incidence of breast cancer cell growth, even greater than tamoxifen alone, emphasizing the strength and importance of diet in preventing and reducing cancer (Cover et al.,1998).

Hormone Therapy

Diet is among the great preventative measures for breast cancer, but many therapies exist for when cancer reaches a more critical stage, including surgery, chemotherapy, hormonal therapy, and radiation therapy. Luminal A is a subtype of breast cancer that can benefit from hormone therapy. Hormone therapy deprives the body of estrogen and progesterone because they are the hormones breast cancer cells use to grow. Breast cancer cells have proteins on them we call hormone receptors. When estrogen and progesterone go through the bloodstream, they bind to these proteins that trigger an "on switch" to allow the cancer cells to grow. Hormonal therapies try to prevent this binding from occurring, preventing the growth of cancer cells (Figure 2).

Hormone therapy is usually started after the removal of breast cancer tissue, considering the health of the patient (MD Anderson Cancer Center & Wendler, 2022). As mentioned, tamoxifen is a medication that binds to protein receptors so estrogen cannot. When tamoxifen is bound to these hormone receptors in place of estrogen, there is no signal triggered that allows the cells to grow in the G1 phase or duplicate in the S phase (Piezzo et al., 2020). This is how tamoxifen helps the body combat growing breast cancer (Figure 2).

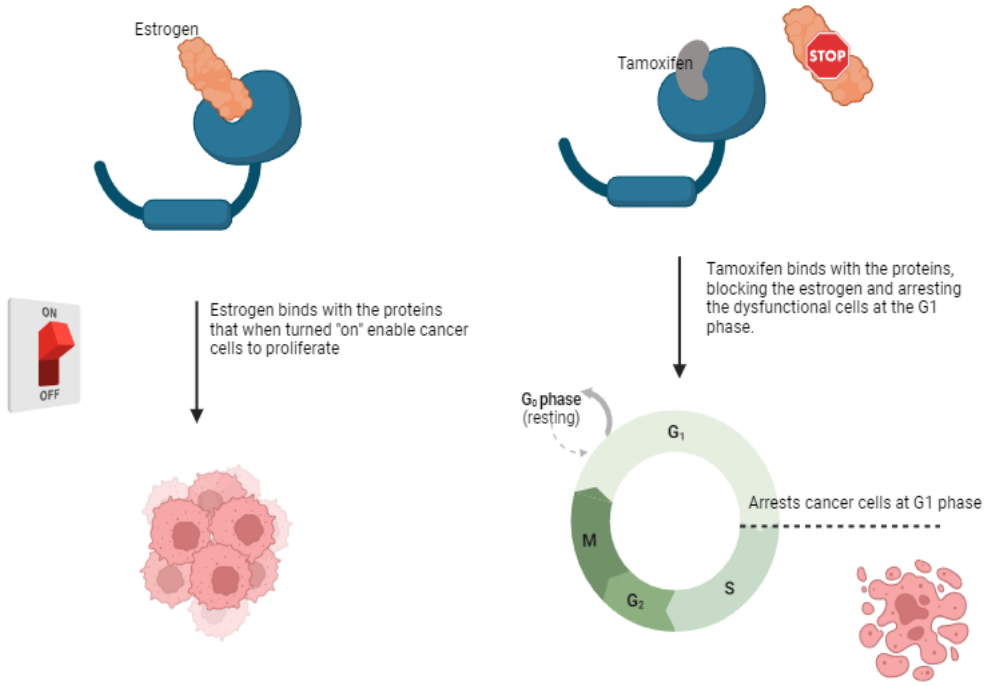

Figure 2. Estrogen vs Tamoxifen binding with the protein – to proliferate or arrest cells in the G1 phase. Created on Biorender.

Although tamoxifen deprives breast cancer cells of estrogen, it simultaneously stimulates estrogen receptors in the bone (Farrar et al., 2023). Women who are under treatment for cancer that go through menopause or are postmenopausal often have a decrease in bone strength and density, and by stimulating estrogen receptors, tamoxifen helps to strengthen the bone.

As mentioned before in the context of Paget's seed and soil theory, breast cancer is most likely to develop bone metastasis. When cancer appears in the bone, the bone grows much weaker and is highly susceptible to fractures. With knowledge of bone metastases, one can suspect that tamoxifen is beneficial in reducing or preventing bone metastasis by keeping the bone healthy while also preventing the growth of breast cancer.

Discussion

In a women's lifetime, she has a 1 in 8 chance of being diagnosed with breast cancer. After any diagnosis of cancer, extremely rigorous treatments need to be enacted in order to have a chance at eradicating the cancer. Cancer is an extremely unpredictable and fast acting disease, especially in the breast, where being unaware of estrogen and progesterone levels can be dangerous. In this paper, a detailed view of the cell cycle was able to give perspective with how estrogen works within the cell cycle to either proliferate or arrest the production of cancer cells. Birth control, late full-term pregnancy, and obesity, among other circumstances can increase a women's risk of getting breast cancer, as well as hereditary genes like BRCA1 & BRCA2. This is why it is important for women be aware of their estrogen and progesterone levels and how to implement every-day, sustainable, healthy practices that can prevent breast cancer. Between the various types of breast cancer - Luminal and Triple Negative breast cancer – and the possibilities of it spreading into the bone, it is overwhelming for patients to deal with treatments and diagnosis, both physically and mentally. That is why prevention is so important. Prevention through healthy eating by incorporating vegetables rich in Indole 3 Carbinol, as well as having a healthy, active lifestyle, can go farther than we think.

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