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Obesity and breast cancer:

Linking inflammation to aromatase activity in cancer progression and therapy resistance

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Layman’s summary

Breast cancer affects an increasing number of women every year. A major risk factor for breast cancer is obesity. I looked into one specific type of breast cancer, which is called ER+ and needs estrogen to grow. In this cancer, I investigated what effects being obese would have on disease progression. First of all, when you are obese you consume a lot more energy than you need. All of the extra energy and nutrients you consume could help the tumor, as it grows very quickly and has high energy demands. At the same time, all the fat tissue that you accumulate in order to store this extra energy supplies the tumor with hormones and growth factors. The second thing that I found out was that when you are obese, you have a chronic state of inflammation in your body. The tumor prefers to have inflammation in its environment, because that makes the surrounding area easier to manipulate so the tumor can hide from the immune system, grow blood vessels, invade other tissues, and eventually spread to other organs. A specific type of immune cell, namely macrophages, in the tumor environment seems to produce a compound called PGE2. This compound stimulates the activity of an enzyme called aromatase, which is responsible for the synthesis of estrogens. Based on my findings, I believe that obesity helps the cancer to have an inflammatory environment full of macrophages. These macrophages then release PGE2, which stimulates aromatase to synthesize more estrogens. The tumor can use this estrogen to grow and the cancer can progress to a more advanced stage. Taking this mechanism and the knowledge around obesity as a risk factor for cancer into account, we can try to think of new treatments for ER+ breast cancer. At the moment, people usually receive a primary treatment like surgery and/or chemotherapy, and an additional treatment, which would be hormone therapy. This hormone therapy then for example targets aromatase in order to disrupt the synthesis of estrogens and prevent the cancer from growing. Unfortunately, many women are already resistant to this form of therapy or become resistant over time, which can lead to them having a relapse in the future. This means that we need to do more research to understand the mechanisms behind treatment resistance better. However, we also need to come up with innovative new therapies or ways to combine multiple different therapies. Additionally, we should try to include someone’s weight into the risk assessment for breast cancer patients, or find other factors outside of weight that can help us to predict inflammation that puts someone at an increased cancer risk.

Abstract

Breast cancer is the most common female cancer and is influenced by a variety of hormonal, genetic, and environmental factors. The most prevalent subtype is ER+, where the cancer relies on estrogen signaling for growth. In this subtype, obesity is a large risk factor, as adipose tissue has several pro-tumorigenic functions. This review aimed to investigate the influence of obesity on cancer progression and its link to inflammation, as well as how inflammation might potentially mediate estrogen signaling. The results showed that the obese diet promotes a dysregulated metabolism, which supports several hallmark properties of cancer such as proliferative signaling via insulin and estrogen. Additionally, adipocytes near the tumor provide inflammatory cytokines and remodeling factors that enable invasion and metastasis. Inflammation is mediated differently in the tumor and the microenvironment. In the tumor, inflammation is kept at a low level and any immune response is being suppressed. Tumor-associated macrophages mediate immune evasion, angiogenesis, as well as invasion and metastasis. The tumor microenvironment is however highly inflammatory, especially in obesity where dying adipocytes form crown-like structures that have a wide range of pro-tumorigenic functions. Literature suggests that PGE2, a compound released by macrophages near the tumor, activates the transcription of the enzyme aromatase. This enzyme participates in estrogen synthesis and subsequent signaling. PGE2 is only the most likely candidate for providing a connection between inflammation and estrogen signaling and its release by macrophages may be regulated upstream by adipokines like leptin. Future research must focus on better understanding the mechanisms behind resistance to treatments with aromatase inhibitors and needs to investigate how we can use our knowledge about obesity as a breast cancer risk factor in clinical practice.

Introduction

Obesity is linked with an increased risk of 13 types of cancer, among them breast cancer (Brown, 2021). Not only does the diet of an obese person contribute excess energy and metabolic building blocks, but there is elevated hormone and growth factor signaling as well as a low level of chronic inflammation that support tumor growth and progression (Park et al., 2014). Especially in breast cancer, obesity appears to have two key roles. First of all, obese people have a higher amount of adipose tissue, which is known to have an endocrine function, and a higher activity of aromatase, an enzyme participating in the biosynthesis of estrogen (Gerard & Brown, 2018). The majority of breast cancers are endocrine-sensitive, meaning that they rely on hormone-signaling for growth and replication (del Re et al., 2012). Specifically, breast cancers are categorized depending on their hormone receptor status, being estrogen and progesterone positive or negative. They are also categorized based on the presence of the HER2/neu protein, which in excess amounts (HER+) can allow cancer cells to grow rapidly (Razavi et al., 2018). Two thirds of breast cancer cases are estrogen-dependent and therefore possess estrogen receptors (ER+). When they are deprived of this hormone, their growth is stunted and disease progression is perturbed (del Re et al., 2012; Gerard & Brown, 2018). As estrogen is essential for this type of breast cancer, this provides an avenue for therapy. While there are also breast cancers that are not estrogen-sensitive (ER-), which are more difficult to treat and more aggressive, the focus of this review will be on the more common estrogen-dependent (ER+) breast cancer, as it is uniquely sensitive to changes in the microenvironment that occur in obese patients (del Re et al., 2012). Besides increasing hormonal signaling, there is a second role for obesity in breast cancer development and progression. While the tumor itself is an anti-inflammatory environment and full of tumor-associated macrophages (TAMs) that contribute to key aspects of tumor progression such as proliferation, angiogenesis, survival, and the suppression of the anti-tumor response, the microenvironment surrounding the tumor is highly inflammatory (Lewis & Hughes, 2007; Constantinou & Fentiman, 2013).
In obese people, the chronic low-grade inflammation present can provide this microenvironment for the tumor. It is suspected that the inflammation leads to activation of signaling pathways beneficial for tumor growth, alters the availability of various metabolites, and remodels the extracellular matrix (ECM) to provide a stiff environment that is favored by progressing tumors (Faria et al., 2020; Wang et al., 2012; Brown, 2021). While high levels of estrogen signaling and a pro-inflammatory microenvironment are two important processes in ER+ breast cancer, few researchers have tried to connect them. A handful of studies have suggested a potential link between macrophages and upregulation of aromatase activity, resulting in increased estrogen signaling (Gerard & Brown, 2018; Samarajeewa et al., 2013; Faria et al., 2020; Wang et al., 2012). In this review I will first elaborate on the estrogen-mediated mechanism of ER+ breast cancer growth and then explain the influences of the obese microenvironment on cancer progression. Next, I will describe how immunity and inflammation is regulated in and around the tumor, and how obesity may influence this regulation. Lastly, I will discuss a mechanism linking inflammation and estrogen signaling, in order to explain how these two aspects may work together in ER+ breast cancer. I hypothesize that one of the compounds released by macrophages in the tumor microenvironment promotes aromatase activity, thereby leading to increased synthesis of estrogens. In the final part of this review I will discuss how these findings influence therapeutic interventions and specifically the issue of treatment resistance that arises in endocrine sensitive breast cancer, and explain avenues for future research.

Upregulated estrogen signaling drives ER+ breast cancer development

Estrogen signaling is a crucial mechanism for tumor growth in a majority of breast cancers, and forms the most common target for treatment. All forms of breast cancer are grouped into categories based on the existence of nuclear hormone and growth factor receptors (del Re et al., 2012; Tower, Ruppert & Britt, 2019). Specifically, they are categorized as receptor positive if either the estrogen receptor (ER+), progesterone receptor (PR+) or human epidermal growth factor receptor 2 (HER2+) are present. Any combination of receptors can co-exist, and receptor positivity in general is favorable as it provides a target for treatment (del Re et al., 2012; Tower, Ruppert & Britt, 2019). Subsequently, triple negative breast cancer, which has none of these receptors, is more difficult to treat. Estrogen signaling is particularly important, as more than three quarters of breast cancers are ER+ and thereby estrogen-dependent (del Re et al., 2012).

The synthesis of estrogen in premenopausal women occurs in the ovaries, while in postmenopausal women synthesis is done through conversion of circulating androgens in different peripheral tissues (del Re et al., 2012). It is however important to note that in the ovaries, androgens are also produced first, and conversion to estrogens is a final step. In both pre- and postmenopausal women, the conversion of androgens into estrogens starts with the cytochrome P450 dependent enzyme aromatase (CYP19A1), which receives electrons from NADPH-cytochrome P450 reductase (del Re et al., 2012). This causes it to catalyze three consecutive hydroxylation reactions, turning a nineteen-carbon androgen like androstenedione or testosterone into an eighteen-carbon estrogen like estrone or estradiol (figure 1a). Aromatase is expressed in many tissues like the breast, fat, muscle, nerves, and skin, but its activity and mRNA expression are increased in breast cancer cells but especially the stromal cells surrounding a tumor (del Re et al., 2012).

ER is a transcription factor belonging to the nuclear receptor superfamily that responds to estrogens and growth factors. When activated, ER can exert its effects on target genes by directly binding estrogen response elements on DNA, or indirectly through protein-protein interactions with other transcription factors such as AP-1, which subsequently binds DNA through its own AP-1 response elements (Jameera Begam et al., 2017). The activation of ER usually depends on the presence of its ligand, which upon binding causes a conformational change in ER (figure 1b). Subsequently, ER dissociates from its chaperones and dimerizes, thereby activating its transcriptional domain (Jameera Begam et al., 2017). However, ER and thus estrogen-mediated gene activation can also be activated ligand-independently (figure 1b). The ER is then activated by crosstalk with growth factor signaling pathways. Ligand-activated growth factor receptors (GFRs) undergo phosphorylation and dimerization and thereafter can associate with members of either two different signaling pathways (Ma et al., 2015). For one, dimerized GFRs can associate by themselves or through recruitment of an adapter protein with p85, the regulatory subunit of PI3K. This connection causes p85 to release its inhibition of the catalytic subunit p110, activating PI3K (Ma et al., 2015). PI3K then catalyzes the conversion of phosphatidylinositol (PIP2) to phosphatidylinositol triphosphate (PIP3), thereby activating AKT. This causes further downstream activation of other components of the PI3K/AKT/mTOR pathway, which supports cancer growth and proliferation (Ma et al., 2015). Alayev et al. (2016) found that

AKT as well as an effector of the downstream mTORC1 have the ability to phosphorylate ER, causing its dissociation from chaperones, dimerization, and subsequent activation (figure 1b). The second pathway that is activated by growth factor signaling is related to the MAP kinase, which is known for its ability to activate transcription factors (Ma et al., 2015). The activated, dimerized GFRs associate with a complex of three proteins (SOS, SHC, GRB2) that catalyzes the conversion of RAS-GDP into RAS-GTP (Ma et al., 2015). RAS-GTP not only also supports the activation of PI3K by interacting with its catalytic domain p110, but it also leads to activation of RAF, MEK, and further downstream MAPK (Ma et al., 2015). The MAP kinase is responsible for phosphorylating ER and thereby causing its activation as previously described (figure 1b). To recapitulate, ER can either be activated through binding of its ligand, or by the action of GFRs. In the latter case, GFRs promote either PI3K/AKT/mTOR and/or MAPK signaling, which in turn lead to the phosphorylation and subsequent activation of ER (Ma et al., 2015).

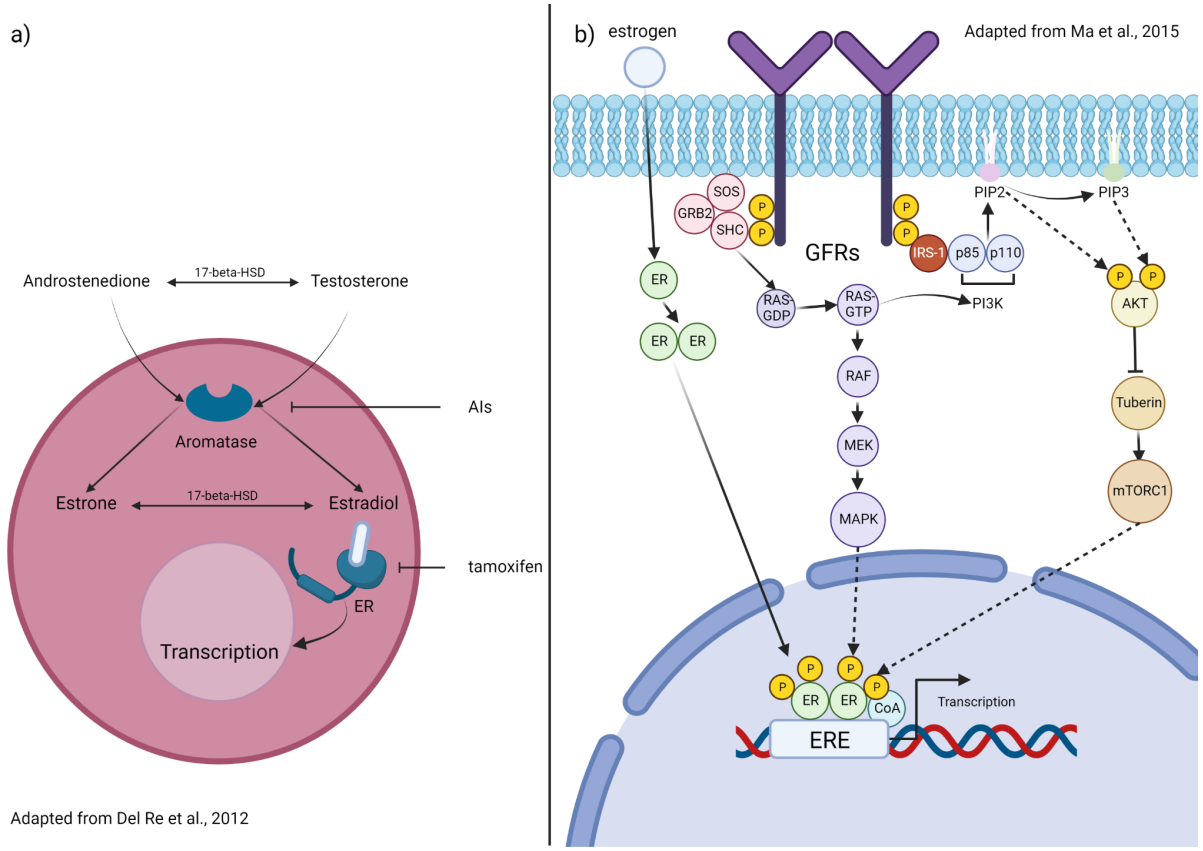

Figure 1. Illustration of estrogen synthesis and signaling. a) Aromatase is responsible for the conversion step of androgens into estrogens, which can then ligand-activate ER. b) On the left we can see ligand-activation of ER by estrogens (estradiol) with subsequent release of ER, dimerization, and activation. It binds to estrogen response elements on the DNA together with coactivators to promote target gene transcription. The remainder of the illustration shows GFR-mediated activation of ER in the absence of estrogen. Downstream effectors of both the PI3K/AKT/mTORC1 or MAPK pathways can phosphorylate and thereby activate ER, which can similarly cause it to dimerize and become active.

Estrogen signaling is associated with cell cycle regulation and proliferation. ER-mediated changes in gene expression appear to target cell cycle regulators such as cyclin D1 (Ma et al., 2015). Cyclin D1 then activates cyclin-dependent kinases 4 (CDK4) and 6 (CDK6) to phosphorylate RB, thereby releasing RB's inhibition of the E2F transcription factors. These transcription factors subsequently activate the expression of genes related to the G1 to S

phase transition, effectively allowing the cell cycle to progress more quickly and with limited control (Ma et al., 2015). However, the estrogen signaling also has an effect on cancer cell proliferation, as the activated ER is coupled to G proteins that can transmit signals from the outside to the inside of the cell. These G proteins can activate a variety of signaling pathways, as the ER subsequently interacts with signaling molecules like PI3K, MAPK, AKT, p21ras and PKC (Liu, Ma & Yao, 2020). Not only do many of these molecules act in pathways promoting proliferation, but some of them also contribute to the phosphorylation and thus further activation of the ER, as previously shown (Liu, Ma & Yao, 2020; Ma et al., 2015). Liu, Ma & Yao (2020) also describe that ER further upregulates PI3K/AKT/mTOR and MAPK signaling via its activation of growth factors such as human epidermal growth factor receptor 2 (hEGFR2) and epidermal growth factor receptor (EGFR), and propose further targets such as the insulin-like growth factor 1 receptor (IGF1R), a part of the IGF pathway involved in cancer growth. Taking together the knowledge about estrogen signaling, we can now conclude that the high expression of ER allows for the acquisition of two hallmark properties of cancer: The first is the evasion of growth suppression through cell cycle manipulation. The second is the maintenance of proliferative signaling by manipulating GFRs and a variety of signaling pathways (del Re et al., 2012; Hanahan & Weinberg, 2011).

The obese microenvironment provides the ideal conditions for cancer progression

The tumor microenvironment present in obese patients contributes to cancer progression. The excess energy intake of an obese person is beneficial for the tumor, because the quickly proliferating cancer cells have an increased metabolic need compared to healthy cells (Park et al., 2014). Irrespective of oxygen availability, cancer cells preferentially use glycolysis for energy and skip the time-consuming yet more energy-efficient process of oxidative phosphorylation in the mitochondria (Hanahan & Weinberg, 2011; Ward & Thompson, 2012). This dysregulation of cellular metabolism, also known as the Warburg effect, is necessary for cancer cell survival and is an emerging hallmark of cancer. Obesity supports this property by providing a greater amount of energy than necessary, which can be converted and stored in adipose tissue (Park et al., 2014; Hanahan & Weinberg, 2011). Cancer cells however do not only require glucose, but they also rely on fatty acids. Breast, ovarian, gastric and prostate tumors all show a high expression of CD36, the fatty acid translocase (FAT) , and remodeling of fatty acid metabolism has been suggested as a mechanism in multiple forms of cancer (Koundouros & Poulogiannis, 2020). Specifically, receptor positive breast cancers, among them ER+ breast cancer, show a different lipid metabolism when compared to triple negative breast cancer. The three aspects that are increased in these cancers are the mobilization, synthesis and oxidation of fatty acids (Koundouros & Poulogiannis). First of all, mobilization of fatty acids in this case can be from surrounding adipose tissue, which breaks down and releases fatty acids (Koundouros & Poulogiannis, 2020). Secondly, the de novo synthesis (lipogenesis) of fatty acids from carbohydrate and amino acids normally only occurs in hepatocytes and adipocytes, but may be reactivated in some cancers. This might occur even in the presence of exogenous lipid sources. Lastly, in periods of high cellular stress, accumulated lipid droplets can undergo beta oxidation in order to eventually serve as a source of energy in the form of ATP and other metabolites such as NADPH (Koundouros & Poulogiannis, 2020). Fatty acids may also be important for cancer progression, as metastatic cells preferentially mobilize in adipose tissue near the mammary gland and visceral

omentum. Adipose tissue can provide the tumor with a variety of growth factors, cytokines and free fatty acids through lipolysis, and fatty acids also appear to play a role in the activity of a range of signaling molecules that also regulate cancer metabolism (Koundouros & Poulogiannis, 2020). Overall, it can be concluded that obesity promotes a dysregulated cellular metabolism, an emerging hallmark of cancer, which allows the tumor to survive and grow (Hanahan & Weinberg, 2011).

The diet of an obese person additionally leads to dysregulation of insulin signaling. The excess intake of energy warrants the compensatory release of insulin from the beta cells of the pancreas. The constant high insulin levels (hyperinsulinemia) in response to high blood glucose levels (hyperglycemia) can however lead to receptors becoming resistant to insulin and subsequently cause the development of type II diabetes and other health consequences of obesity (Calle & Kaak, 2004; Arcidiacono et al., 2012). The excess insulin contributes to signaling by binding the insulin receptor, causing transcription of target genes that suppress apoptosis and increase cellular proliferation (Calle & Kaaks, 2004). At the same time, insulin can reduce the synthesis of insulin-like growth factor binding proteins 1 and 2 (IGFBP1, IGFBP2). This increases the bioavailability of insulin-like growth factor 1 (IGF1), which through its receptor IGF1R can similarly affect target genes to prevent apoptosis and support proliferation (Calle & Kaaks, 2004). Insulin signaling also participates in hormone-mediated tumorigenesis, as it downregulates the levels of sex-hormone binding globulin (SHBG). This increases estrogen bioavailability and thereby supports estrogen-mediated growth signaling in the tumor (Arcidiacono et al., 2012). Arcidiacono et al. (2012) provide an overview of the connection between insulin resistance and cancer risk, also in connection with obesity, which is outside of the scope of this review. However, what is important to highlight here is that the changes in metabolism directly lead to the acquisition of one of the hallmarks of cancer, being the maintenance of proliferative signaling (Hanahan & Weinberg, 2011). Examples of this are insulin and IGF1 signaling, as well as estrogen signaling in response to increased availability of estrogen due to insulin-mediated targeting of SHBG.

The excess adipose tissue as a result of a high energy intake has several pro-tumorigenic functions. Adipose tissue is an important microenvironmental component in breast cancer, as the stroma of the mammary gland is directly embedded in the mammary fat pad (Wang et al., 2012). This environment primarily consists of fibroblasts and adipocytes as well as their progenitors. However, there are also vascular, endothelial and lymphatic cells, as well as macrophages present. Both fibroblasts and adipocytes are microenvironmental components that can be restructured to allow invasion and metastasis (Wang et al., 2012; Park et al, 2014). It has been known since 1992 that breast cancer is able to grow better in fat tissue than other environments (Elliott et al.). Mature adipocytes in the vicinity of the tumor invasive front are called cancer-associated adipocytes (CAAs). They undergo a range of phenotypic and functional changes as cancer progresses (Wang et al., 2012; Park et al, 2014). In a co-culture model mimicking the adipocyte-cancer cell crosstalk, Wang et al. (2012) found that adipocytes near murine breast cancer cell lines lost their lipid content, de-differentiated, and overexpressed inflammatory cytokines and proteases. This shows that paracrine signals from cancer cells can induce lipolysis in adipocytes, with the subsequent increase in free fatty acids causing an increase in inflammation and structural remodeling (Park et al., 2014). CAAs support multiple hallmarks of cancer through their phenotype. For one, their release of free fatty acids contributes to the dysregulated metabolism in cancer, which is an emerging hallmark (Hanahan & Weinberg, 2011; Wang et al, 2012; Park et al, 2014). Second, their

de-differentiation is supported by multiple extracellular matrix (ECM) remodelers such as the protease matrix metalloproteinase 11 (MMP11) and the high expression of collagen VI. These structural changes support the epithelial to mesenchymal transition (EMT), which is the change in epithelial cells to lose their polarity and adhesion while gaining mobility and invasiveness. The EMT also characteristically occurs shortly before invasion and metastasis, which are further cancer hallmarks (Wang et al, 2012; Hanahan & Weinberg, 2011). Third, the rapid, hypertrophic expansion of adipose tissue in obesity is similar to tumor growth as it induces hypoxia, which can lead to resistance to the primary cancer treatment chemotherapy. The hypoxia then triggers compensatory angiogenesis in the adipose tissue. Angiogenesis is another hallmark of cancer which allows the tumor to have sufficient blood supply in its surrounding area (Park et al., 2014). This attempt to overcome the limited nutrient and oxygen availability causes the expression of the transcriptional regulator HIF-1-alpha, as well as upregulation of ECM proteins like collagens, MMPs, and monocyte chemoattractant protein 1 (CCL2/MCP1) (Park et al., 2014). Besides CAAs, the local stem cell population, namely adipocyte-derived stem cells (ADSCs), participate in the release of various molecules promoting invasion and EMT. Via tumor-mediated TGF-beta signaling, the ADSCs can be differentiated into cells that resemble cancer-associated fibroblasts (CAFs, see box 1), possibly contributing to the dense fibroblast composition at the center of breast tumors (Wang et al., 2012). It can now be summarized that CAAs clearly contribute to several hallmarks of cancer and form a large part of the reason that obesity increases the risk of cancer and supports its progression.

Text Box 1. Further information about the role of CAFs in the tumor microenvironment.
Similar to CAAs, CAFs are altered fibroblasts that support the tumor by changing the environment. Through the application of mechanical force, the cross-linking of enzymes or the action of proteases, CAFs alter the structure of the ECM through collagen-mediated pathways that either block or guide the movement of migrating cancer cells (Park, Sahai & Rullan, 2020). Through their alterations of the ECM, as well as the release of a variety of growth factors and cytokines, they can induce angiogenesis, proliferation, migration, EMT, and metastasis. Lastly, they are also able to exert immunosuppressive functions by regulating macrophage recruitment and T cell polarization (Park, Sahai & Rullan, 2020; Prakash, 2016). Within CAFs, heat shock factor 1 (HSF1) is frequently highly expressed. This transcription factor is responsible for eliciting a different genetic program in the stroma compared to the tumor, and thereby enriches pathways related to angiogenesis, ECM organization, adhesion and migration in the microenvironment (Scherz-Shouval et al., 2014). All of this shows that the CAAs and CAFs present in adipose tissue play a key role in establishing an environment that supports the tumor.

The regulation of immunity and inflammation near the tumor supports cancer progression and is influenced by obesity

While estrogen signaling allows the ER+ breast cancer to proliferate and continuously grow, the interactions of the tumor with the patient's immune system are key to cancer progression (Tower, Ruppert & Britt, 2019). It is hypothesized that the immune system both protects the patient and also stimulates the tumor through its response to a developing cancer ("immunoediting") (Tower, Ruppert & Britt, 2019). After immunosurveillance detects an initiating cancer, a response is mounted by the innate and adaptive immune system to eliminate all tumor cells (Tower, Ruppert & Britt, 2019). Any remaining cancer cells subsequently mutate in order to be less recognizable by the immune system. The tumor cells exist in equilibrium with their microenvironment and utilize immunosuppressive tactics

in order to escape the immune system's control (Tower, Ruppert & Britt, 2019). Avoiding destruction by the immune system is an emerging hallmark of cancer that is key for disease progression as it essentially enables a tumor to form and expand without being destroyed by the patient's body (Hanahan & Weinberg).

The tumor itself is an anti-inflammatory environment, where immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells (MDSCs) are present (Hanahan & Weinberg, 2011; Constantinou & Fentiman, 2013). Around 50% of the immune cells are tumor-associated macrophages (TAMs), which are pro-tumorigenic (Lewis & Hughes, 2007; Constantinou & Fentiman, 2013). An active, adaptive immune response marked by the presence of cytotoxic T cells (CTLs) and helper T cells (Th) releasing tumor-limiting cytokines, does not seem to be important for patient survival in ER+ breast cancer. Unlike other subtypes, ER+ breast cancer is less immunogenic and thus less likely to trigger an anti-tumor immune response in the patient body (Constantinou & Fentiman, 2013; Tower, Ruppert & Britt, 2019).

The most important players are TAMs, which support cancer progression and angiogenesis, and have been linked to reduced patient survival (Lewis & Hughes; Constantinou & Fentiman, 2013). Already in 2007 it was shown that the disruption of macrophage growth would delay angiogenesis and cancer progression (Lewis & Hughes, 2007; Constantinou & Fentiman, 2013). TAMs are created from other macrophages. First, circulating monocytes are diverted to migrate into cancer tissues in response to chemoattractants like monocyte chemoattractant protein 1 (MCP-1). Then, they differentiate into macrophages, specifically TAMs (Constantinou & Fentiman, 2013). Mature TAMs are functionally and phenotypically similar to the alternatively activated M2 macrophages, which have an immunosuppressive function (Constantinou & Fentiman, 2013). TAMs and M2 macrophages mainly differ in their metabolism, as TAMs rely on glycolysis for energy and release large amounts of lactate, while M2 macrophages rely primarily on oxidative phosphorylation (de-Brito et al., 2019; Constantinou & Fentiman, 2013). These differences can be explained by the conditions in the tumor environment. As oxygen is not always readily available, an anaerobic metabolism appears favorable (San-Millan & Brooks, 2017). On the other hand, this type of metabolism including the production of lactate as a mediator of carcinogenesis, also known as the Warburg effect, is common in cancer and preferred even in the presence of ubiquitous amounts of oxygen (San-Millan & Brooks, 2017).

TAMs have a variety of roles, but the three that stand out will be discussed here. For one, they suppress anti-tumor immunity by releasing cytokines like IL-10 and factors like indoleamine-2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2), as well as suppressing the release of proinflammatory cytokines like IL-12. On the other hand, they also express higher levels of the hypoxia-induced transcription factors 1 alpha and 2 alpha (HIF1-alpha, HIF2-alpha), through which they appear to regulate the expression of various proangiogenic factors such as vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP9) or basic fibroblast growth factor (bFGF) (Zhang et al., 2016). Lastly, TAMs also participate in invasion and metastasis by releasing the chemokine C-C motif ligand 18 (CCL18) which induces the epithelial-mesenchymal transition (EMT) through the PI3K/AKT/GSK3-beta/SNAIL pathway (Zhao et al., 2020). It is unclear how the re-education of macrophages into TAMs takes place, but it is suggested that it may be regulated via certain cytokines and chemokines. Several authors suggest that transforming growth factor

beta (TGF-beta), released for example by MDSCs, induces non-activated macrophages to become TAM-like and acquire an M2 phenotype through an unknown mechanism (Zhang et al., 2016; Tower, Ruppert & Britt, 2019). Additionally, interleukins like IL-10 released by other immune cells such as T helper cells have been suggested to play a role in differentiating macrophages into TAMs. Currently, a variety of interleukins, chemokine, and TGF-beta are likely important for TAM polarization (Tower, Ruppert & Britt, 2019; Ding & Ge, 2020). Overall, TAMs appear to be a key player in the normally not highly immunogenic ER+ breast cancer, as they regulate multiple additional hallmarks of cancer according to the criteria of Hanahan and Weinberg (2011), namely inducing angiogenesis, activating invasion and metastasis, and avoiding immune destruction.

While the tumor suppresses immunity and inflammation, the surrounding microenvironment is highly inflammatory and supports cancer progression in different ways (Gerard & Brown, 2018; Constantinou & Fentiman, 2013; Brown, 2021). Especially obese people have chronic levels of low-grade inflammation throughout their body and locally near the tumor (Gerard & Brown, 2018; Brown, 2021). This inflammatory environment has been recognized as an enabling characteristic of cancer, supporting various hallmark properties (Hanahan & Weinberg, 2011). The source of the inflammation are necrotic adipocytes that are surrounded by macrophages attempting to clear them from the area. This formation is also called a crown-like structure (CLS), and is found in around half of breast tissue from women undergoing mastectomy (Wang et al., 2012; Iyengar et al., 2016). Normal breast tissue obtained from the same women shows macrophage infiltration as well as high expression of inflammatory cytokines IL-6 and IL-8 and chemokines such as CCR5 (Wang et al., 2012). It is important to note that white adipose tissue (WAT) inflammation in the breast occurs in 90% of obese women, creating an increased risk of cancer development and progression. The patients with a high WAT inflammation also show higher levels of insulin, glucose, leptin, triglycerides, C-reactive protein and IL-6, as well as lower levels of adiponectin and HDL cholesterol (Iyengar et al., 2016). WAT inflammation can partially explain the connection between metabolic syndrome and a worse outcome for breast cancer patients. High levels of IL-6 and C-reactive protein are associated with shortened overall and disease-specific survival, while the elevated leptin to adiponectin balance is associated with adverse outcomes such as high proliferation and cancer cell survival. The macrophages inside CLS are not TAMs, but rather regular adipose tissue macrophages of the type M1 that participate in establishing an inflammatory environment (Engin et al., 2019). The CLS assist in a variety of processes supporting the tumor, such as the release of pro-inflammatory cytokines, mTOR signaling, insulin signaling, oxidative stress, hypoxia and compensatory angiogenesis, and the synthesis of estrogens by upregulating aromatase (Engin et al., 2019). Aromatase is naturally expressed in the ovaries and peripheral tissues such as adipose tissue, but also in the tumor. In the case of excess adipose tissue in obesity, aromatase may be expressed at an even higher level (del Re et al., 2012). It is hypothesized that CLS supports aromatase activity through one of the following ways. Cytokines and chemokines released by CLS like TNF-alpha may activate aromatase, but also cross-talk with the signaling molecule AKT or the inflammatory NF-kappa-B pathway are suspects (Engin et al., 2019). Additionally, the hormone leptin that stands in balance with its counterpart adiponectin to regulate hunger and energy intake may play a role. Leptin is highly overexpressed in obese and metabolically unhealthy people (Engin et al., 2019). Leptin has several pro-tumorigenic functions and appears to participate in the polarization of TAMs. The mechanism through which it influences aromatase is unclear, but some

suggestions include interactions with MAPK and STAT3 signaling (Sanchez-Jimenez et al., 2019) and through promoting the release of prostaglandin E2 (PGE2) from macrophages (Delort et al., 2015). Lastly, the enzyme cyclooxygenase 2 (COX-2) present primarily in macrophages and TAMs potentially regulates aromatase activity (Engin et al., 2019), which is described in the following section in greater detail. Overall, we can now see that the distinct regulation of inflammation in or near the tumor contributes to a wide variety of processes allowing for cancer progression. The increased systemic inflammation in obesity may thereby influence the treatability of cancer as it can provide a suitable tumor microenvironment. In order to fully understand the picture of ER+ positive breast cancer, we also need to take into account that inflammation is very likely adding to estrogen signaling, if not exacerbating it.

The release of PGE2 by macrophages causes upregulation of aromatase, indicating a link between immunity and estrogens

Prostaglandin E2, a compound released by macrophages, appears to activate estrogen synthesis by promoting the transcription of aromatase (Gerard & Brown, 2018). PGE2 is a prostanoid which is synthesized through the action of a sequence of enzymes (Finetti et al., 2020). Fatty acids such as arachidonic acid are released from the cell membrane through phospholipase A2 (PLA2) family members. The fatty acids are then oxidized into prostaglandin G2 (PGG2) and reduced to prostaglandin H2 (PGH2) (Finetti et al., 2020). These steps are executed by the cyclooxygenase (COX) enzymes 1 and 2. Finally, the microsomal PGE synthases 1 and 2 (mPGES-1, mPGES-2) and the cytosolic PGE synthase (cPGES) undertake the final step to PGE2 (Finetti et al., 2020). The expression of several of these enzymes is altered in cancer. For one, while COX-1 is constitutively expressed in healthy cells, COX-2 expression is highly limited and only upregulated during inflammation and in cancer (Finetti et al., 2020). Both in premalignant lesions and advanced cancers, PGE2 has been found to be elevated, and the high expression of COX-2 and accumulation of PGE2 is associated with a poor outcome (Gerard & Brown, 2018; Basu, Rossary & Vasson, 2016). Additionally, the expression of mPGES-1 is elevated in cancer. Through signaling with the prostaglandin E2 (EP) receptors, PGE2 has been speculated to play a role in proliferation, invasion, EMT, angiogenesis, and the downregulation of apoptosis, as well as inflammatory NF-kappa-B signaling, but expression of the EP receptors varies greatly by cell type and location (Finetti et al., 2020).

The release of PGE2 by macrophages may be responsible for upregulated aromatase activity near the tumor. COX-2 is especially overexpressed in TAMs, mediating their release of PGE2 and the cytokine IL-6. The latter is important because IL-6 can induce MMP-9 (see Kothari et al., 2014), an important promoter of EMT in breast cancer cells, and it helps to upregulate the expression of COX-2 and mPGES-1 in surrounding tumor cells, while reducing the expression of PGE2 inhibitory enzymes (Ding & Ge, 2020; Finetti et al., 2020). A high level of PGE2 also seems to induce polarization of macrophages into the M2-like phenotype. PGE2 generally affects a wide variety of immune cells. For one, it induces differentiation, recruitment, and activation of the immunosuppressive MDSCs (Ding & Ge, 2020). PGE2 also decreases the number, migration, and antigen presentation of dendritic cells, and inhibits natural killer cells. Lastly, it promotes further immunosuppression by modifying T cell proliferation and primarily supporting the development of helper T cells of

the anti-inflammatory Th2 type as well as regulatory T cells (Ding & Ge, 2020). In obesity it seems likely that the high availability of free fatty acids in the environment stimulates macrophages near the tumor, primarily TAMs, to express COX-2 and produce PGE2. This PGE2 can then be found in high amounts in the microenvironment and taken up by the tumor, where it then stimulates the expression of aromatase and synthesis of estrogens used for growth signaling.

A multitude of pathways have been proposed that may explain how PGE2 activates the aromatase promoter and thereby affects estrogen signaling (figure 2). For one, binding of PGE2 to its receptors leads to coupling to adenylyl cyclase, which is the enzyme synthesizing cAMP. The production of cAMP and subsequent activation of the cAMP-dependent protein kinase (PKA) leads to the PKA-mediated phosphorylation of the cAMP response element-binding protein (CREB), causing CREB to be translocated to the nucleus (Gerard & Brown, 2018; Samarajeewa et al., 2013). CREB forms a complex with its binding protein CBP and the histone acetyltransferase p300, and together this complex binds at least two separate cAMP response elements (CRE) on aromatase's promoter II, causing its activation. However, PGE2 also appears to stimulate interaction of the CREB-regulated transcription factors 2 and 3 (CRTC2, CRTC3) by supporting their nuclear translocation, which is dependent on cAMP and calcium signaling. For the majority of its target genes, CREB requires its coactivators (CRCTs) to exert its function (Gerard & Brown, 2018; Samarajeewa et al., 2013). Breast adipose stromal cells show a higher expression of CREB as well as the transcriptional regulator HIF-1-alpha. It is important to recall that especially the tumor microenvironment shows increased aromatase activity, supplying estrogens to the tumor (del Re et al., 2012). HIF-1-alpha also binds to promoter II of aromatase and shows increased localization as well as mRNA and protein expression when levels of PGE2 are high (Gerard & Brown, 2018; Samarajeewa et al., 2013). Furthermore, PGE2 inhibits several negative regulators of aromatase such as the AMP-activated protein kinase (AMPK) (Gerard & Brown, 2018). AMPK is a sensor of cellular energy status and is activated by an increase in the AMP:ATP ratio caused by metabolic stress. It exerts its functions through the tumor suppressor liver kinase B1 (LKB1), and the LKB1/AMPK pathway inhibits the entry of CRTC2 into the nucleus (Gerard & Brown, 2018). PGE2 inhibits the expression of LKB1, which leads to reduced activation of AMPK. Subsequently, AMPK cannot phosphorylate CRTC2, which then translocates the nucleus to associate with CREB and increases the expression of aromatase (Gerard & Brown, 2018). Several studies suggest further mechanisms, as for example p53 acts as a negative regulator of aromatase that is inhibited by PGE2. There are also some ideas that the MAPK pathway may regulate aromatase via PGE2, and that the activation of certain protein kinases (PKC, PKA) forms an alternative pathway (Gerard & Brown, 2018; Faria et al., 2020; Wang et al., 2012). The current state of knowledge surrounding PGE2 overall indicates that it is a crucial part of ER+ breast cancer progression, as it mediates anti-tumor immunity and potentially the polarization of TAMs, and through a variety of pathways elevates aromatase activity and thus estrogen signaling.

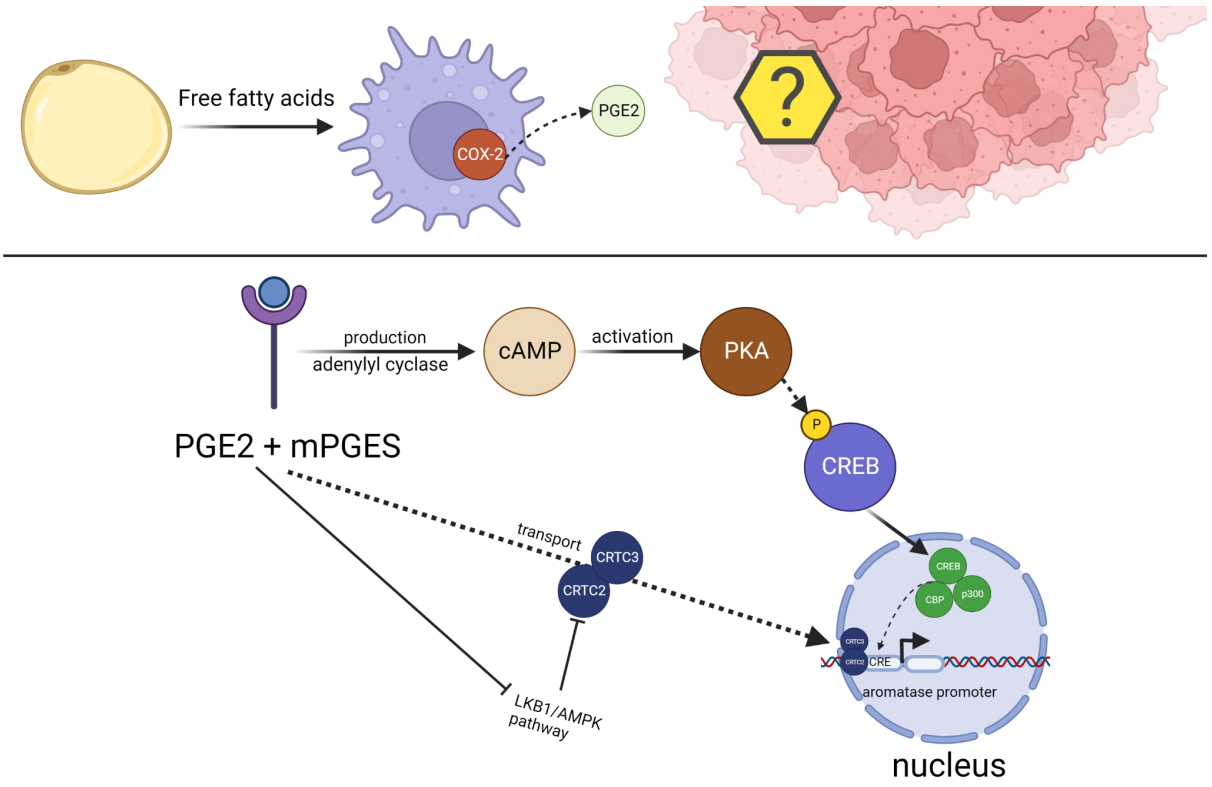

Figure 2. Potential mechanism through which macrophage-mediated release of PGE2 upregulates aromatase. PGE2 binding to its receptor signals adenylyl cyclase to synthesize cAMP, which activates PKA. Then, PKA phosphorylates CREB, which enters the nucleus and forms a complex with CBP and p300, binding the CRE elements near the aromatase promoter. PGE2 also mediates the import of coactivators CRTC2/3 into the nucleus, and inhibits LKB1 of the LKB1/AMPK pathway, releasing the block of CRTC2 import into the nucleus.

On the other hand, this is also related to the fact that the expression of PGE2 is not only in the tumor and TAMs, but PGE2 can also be found in high levels in the surrounding adipose tissue of obese women (Gerard & Brown, 2018; Wang et al., 2012). Malignant cells secrete TNF-alpha and IL-11 among other molecules, which are anti-adipogenic and inhibit the differentiation and maturation into mature adipocytes. TNF-alpha is also one of the factors alongside IL-6 which appear to promote PGE2- mediated upregulation of aromatase (Gerard & Brown, 2018). Additionally, while inflammation may contribute to aromatase activity, there are likely other factors at play, such as the adipokine leptin. This hormone seems to regulate both TAMs and the expression of COX and PGE2, as well as multiple pathways of action that have been proposed for PGE2 (see information box 2). Throughout this review we have seen that WAT inflammation surrounding the tumor allows the establishment of a pro-tumorigenic environment, supports estrogen signaling, and mediates various aspects of disease progression through the release of adipokines. This underlines the point that obesity - and the accompanying systemic inflammation - is a risk factor for breast cancer that should not be underestimated.

Information Box 2. Leptin could regulate inflammation and aromatase activity.

Leptin is an adipokine regulating energy balance together with its counterpart adiponectin. It is present in higher levels in obese people (Engin et al., 2019; Atoum et al., 2020). Through it signaling pathway leptin/Ob-R/STAT3, it achieves long-term constitutive activation of its downstream effectors. This allows it to mediate resistance to drugs like tamoxifen or aromatase inhibitors (Gelsomino et al., 2020). However, leptin also appears to mediate the recruitment of TAMs, stimulate their production of interleukins, and thereby promote invasion and metastasis. Leptin secretion also promotes an M2-like phenotype with increased motility of macrophages (Gelsomino et al., 2020). Leptin has been shown to enhance aromatase transcript expression and there are multiple theories on how that functions. For one, associations between the expression of leptin and the leptin receptor (LEPR) with aromatase as well as MAPK and STAT3 have been demonstrated (Sanchez-Jimenez et al., 2019). On the other hand, leptin may support the expression of PGE2 by macrophages through upregulation of the COX-2 enzyme or suppression of the metabolic regulators LKB1/AMPK. Many other mechanisms such as PKC/MAPK signaling, p53 suppression and HIF-1-alpha have been suggested (Sanchez-Jimenez et al., 2019). Leptin may also affect estrogen signaling through other means unrelated to aromatase, for example by increasing different hormones in estrogen metabolism. Some studies have additionally suggested a direct positive effect on the expression of ER (Delort et al., 2015; Atoum et al., 2020). Leptin furthermore has other pro-tumorigenic functions, as it enhances the production of several pro-inflammatory cytokines, regulates cyclin D1, angiogenesis (VEGF), cell survival (Bcl2), and DNA damage (reactive oxygen species). It also enhances the production of COX and increases in leptin signaling go hand in hand with increased PGE2 expression (Delort et al., 2015).

Discussion and future perspectives for ER+ breast cancer treatments

The insights obtained throughout this review must be taken into consideration when thinking about novel treatments for ER+ breast cancer. At the moment, the issue is not that there are not enough forms of treatment available, but rather that patients quickly become resistant to them. Current therapies for ER+ breast cancer consist of a primary cancer treatment, with adjuvant endocrine therapy focused on disrupting estrogen signaling. Specifically, therapies either disturb ER activity or estrogen synthesis (del Re et al., 2012). The first form of therapy often used are selective estrogen receptor modulators such as tamoxifen. This drug binds to the ligand-binding domain of the ER and causes the dissociation of coactivators and the association of corepressors, inhibiting gene transcription (Jameera Begam et al., 2017 & Del Re et al., 2012). However, in recent years the inhibition of aromatase, the rate-limiting enzyme in estrogen synthesis, has been found to be a more effective target for treatment (del Re et al., 2012). Aromatase is normally responsible for the final step of the estradiol and estrone synthesis. Aromatase inhibitors (AIs) thus directly impede estrogen production, meaning that ligand-activated ER signaling is no longer possible (del Re et al., 2012). AIs subsequently prevent cancer progression via reduced cell proliferation due to lowered estrogen levels available for the activation of estrogen signaling (Jameera Begam et al., 2017). A more detailed explanation of the types of endocrine therapy and their mechanisms can be found in Ma et al. (2015).

While these different treatment modalities are fairly successful, most patients only initially respond to them and some do not respond at all. While premenopausal women primarily derive estrogen from their ovaries, postmenopausal women synthesize it in their peripheral tissues, namely adipose tissue, breast tissue, and skin tissue, through aromatase activity

(Ma et al., 2015; Jameera Begam et al., 2017). In these patients, aromatase converts androstenedione and testosterone from ovaries and adrenal glands to estrone and estradiol respectively. In ER+ breast cancer of postmenopausal women, the aromatase is highly expressed in both the peripheral tissues and intratumorally (Ma et al., 2015). This has two effects. For one, if these postmenopausal women are obese, excess adipose tissue can provide high levels of aromatase and thus estrogen signaling. On the other hand, treatment with aromatase inhibitors can effectively reduce estrogen production, which is not possible to the same extent in premenopausal women without suppressing the function of their ovaries, ablating them or removing them (Ma et al., 2015). This means that treatment success may depend on a patient's age. At the same time, treatment resistance is something that can happen to any patient, irrespective of their demographic factors. 15-20% of patients are intrinsically treatment-resistant and another 30-40% acquire resistance over the years (Lei et al., 2019). Intrinsic resistance is often only specific to one form of endocrine therapy. This means that if someone is tamoxifen-resistant they may still respond to AIs, and treatment can be continued in order to prevent cancer progression, metastasis and death (Lei et al., 2019). However, it is important to take into account that endocrine therapy is given for several years after the start of treatment, in order to prevent relapse (American Cancer Society, 2021). If the patient acquires resistance in this timeframe, relapse can occur as late as ten or more years after diagnosis (Lei et al., 2019). Seeing as ER+ breast cancer has a high incidence rate and makes up the majority of breast cancer deaths due to the high number of affected patients, advances in endocrine therapy are necessary to improve patient outcomes (Lei et al., 2019).

However, mechanisms of treatment resistance are not well-elucidated thus far. As the focus of this review was on aromatase due to its connection with obesity, this section will also focus on aromatase inhibitors as a form of treatment. Going over all forms of endocrine therapy in detail would be far beyond the scope of this review. Resistance to aromatase inhibitors is not yet understood in much detail, however, two main mechanisms have been described in literature. The first potential mechanism is constitutive activation of ER, which could be achieved through epigenetic modifications, alterations in the binding domains, alternative cofactor recruitment, and other modalities (Hanamura & Hayashi, 2018; Mills et al., 2018). The second suggested mechanism is estrogen-independent growth signaling (Hanamura & Hayashi, 2018). Even in the absence of estrogens, some ER+ breast cancer cells can survive, as they depend on GFRs for signaling instead (Chen et al., 2013). This could for example be through PI3K/AKT/mTOR signaling to regulate proliferation. This pathway may also cause cell cycle dysregulation through downstream targeting of cyclin D1 (Mills et al., 2018; Ma et al., 2015). An alternative explanation could be activation of the IGF pathway through insulin signaling, which also triggers the PI3K/AKT and MAPK pathways through an adaptor protein (IRS-1). The 14fold overexpression of IGF1R in ER+ breast cancer cells makes this hypothesis equally likely (Arnedos et al., 2014). There are also other ways through which resistance to AIs can be mediated. For example, Liu et al. (2017) suggest that a higher number of TAMs, which they deduce have been polarized through the Notch/Jagged1 pathway, is associated with aromatase inhibitor resistance. Knowing that TAMs release PGE2, the compound suspected to lead to the upregulation of aromatase, it is possible that they render AIs ineffective. Overall, it's important to remember that a large range of "resistance genes" and pathways have been proposed that all play a role in cancer cell survival and growth, but we lack knowledge on how the escape from treatment is

mediated and possibly epigenetically maintained as therapy resistance arises (Chen et al., 2013).

There is still a lot we have to learn about the underlying mechanisms of ER+ breast cancers. For one, the mechanism of how cancer growth and progression is facilitated through estrogen signaling and inflammation has become a lot clearer. However, further explanations of the upstream mechanisms, for example via leptin, are needed. Additionally, the mechanism for resistance to hormonal therapies such as aromatase inhibitors needs to be better understood in order to be able to successfully treat ER+ breast cancer patients. Currently, when a patient becomes resistant to one type of endocrine therapy, it is possible to use a different class of hormonal drug or a combination of endocrine therapies (Ma et al., 2015). However, seeing as macrophage-mediated PGE2 production promotes estrogen signaling, combination treatments of aromatase inhibitors with COX-2 inhibitors may also be useful. However, this depends on having inhibitors that are specific to COX-2 and not both COX-1 and COX-2, as co-targeting of COX-1 leads to a multitude of side effects (Faria et al., 2020). Additionally, it is important to consider that by targeting aromatase and thus the synthesis of estrogens, we are only targeting the ligand-mediated activation of ER signaling. In order to target the ligand-independent signaling we would need to find a target for safely interrupting growth factor signaling or target signaling pathways like PI3K/AKT or MAPK.

Additionally, seeing as obesity increases the risk of breast cancer, it should be taken into consideration in clinical practice. Park et al. (2014) also report that patients who were obese before they were diagnosed have higher recurrence rates of cancer. Obese patients also have a higher risk of becoming treatment-resistant (Faria et al., 2020). 13% of the global adult population or 650 million people are already obese, and by 2030 a billion people worldwide are projected to be obese (WHO, 2018; World Obesity Federation, 2022). With this increase in obesity, there will also be higher numbers of people suffering from related health conditions such as various types of cancer. New tools will be required to determine whether an obese person is at an increased risk of cancer. Iyengar et al. (2016) suggest not only looking at a patient's BMI, but also analyzing a blood sample to determine the presence of inflammation in white adipose tissue, which is at the root of the increased disease risk. Longitudinal studies are required to determine the predictive power of measurements of white adipose tissue inflammation, adipokine balance or other prognostic values. Future treatments may subsequently focus on reducing inflammation in obese and overweight patients. Faria et al. (2020) suggest supplementation with omega-3 fatty acids and resveratrol, which are compounds that have been found to reduce inflammation in the mammary fat pad. Lifestyle interventions (as described by Brown, 2021) are effective at increasing the metabolic health of obese and overweight patients and could be used preventatively and as a support of cancer treatment. Future research should also demonstrate whether the knowledge gained in ER+ breast cancer is applicable in other hormone-sensitive cancers, such as in the endometrium, where obesity also constitutes a large risk factor.

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