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Breast Cancer

Obesity and Breast Cancer: Molecular Interconnections and Potential Clinical Applications

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Obesity • Breast cancer • Risk factors • Adipokines • Insulin resistance • Polymorphisms

Abstract

Obesity is an important risk factor for breast cancer (BC) in postmenopausal women; interlinked molecular mechanisms might be involved in the pathogenesis. Increased levels of estrogens due to aromatization of the adipose tissue, inflammatory cytokines such as tumor necrosis factor-α, interleukin-6, and prostaglandin E_2, insulin resistance and hyperactivation of insulin-like growth factors pathways, adipokines, and oxidative stress are all abnormally regulated in obese women and contribute to cancerogenesis. These molecular factors interfere with intracellular signaling in the mitogen-activated protein kinase and phosphatydilinositol-3-phosphate/mammalian target of rapamycin (mTOR) pathways, which regulate the progression of the cell cycle, apoptosis, and protein synthesis. In this context, structural defects of typical genes related to both BC and obesity, such as leptin, leptin receptor, serum paraoxonase/arylesterase 1, the fat mass and obesity-associated gene and melanocortin receptor 4, have been associated with a high or low risk of BC development. The early detection of these gene alterations might be useful as risk predictors in obese women, and targeting these pathways involved in the BC pathogenesis in obese women is a potential therapeutic tool. In particular, mTOR pathway deregulation concurs in both obesity and BC, and inhibition of this might disrupt the molecular interlinks in a similar manner to that of metformin, which exerts definite anticancer activity and is currently used as an antidiabetic drug with a weight-reducing property. The identification of both genetic and pharmacological implications on the prevention and management of BC is the ultimate aim of these studies. ***The Oncologist*** 2016;21:1–14

Implications for Practice: Obese women are at risk of breast cancer, but clinicians lack concrete tools for the prevention or early diagnosis of this risk. The present study, starting from the biology and the molecular defects characterizing both obesity and breast cancer, analyzed the potential molecules and genetic defects whose early identification could delineate a risk profile. Three steps are proposed that are potentially achievable in the clinical assessment of obese women, namely the evaluation of altered levels of serum molecules, the identification of genetic polymorphisms, and the study of the transcriptomic profile of premalignant lesions. Finally, the therapeutic implications of this molecular assessment were evaluated.

Introduction

Breast cancer (BC) is the highest incidence tumor and the most common cause of death from cancer in women [1]. Obesity is a known risk factor in postmenopausal women [2, 3] and is present in up to 50% of all BC cases in older women [4]. It has been estimated that by preventing overweight, the annual incidence would be reduced by 50%, to less than 13,000 cases in the European Union [5]. Nevertheless, the BC risk in obesity is different among ethnic groups; the association of an increased body mass index (BMI) with BC appears to be particularly strong among the Asia-Pacific populations [6].

A meta-analysis of observational studies suggested that the risk of BC is 12% higher for each 5 kg/m^2 increase in BMI in postmenopausal women [4]. In contrast, a recent meta-analysis examining prospective cohort and case-control studies showed that obesity exerts minor effects on BC development after data adjustment for age, race, and marital status [7]. Unlike in postmenopausal women, a high BMI is apparently protective in premenopausal women [4], because a U.K. population-based cohort study revealed that, independently of smoking, each 5-unit BMI increase was inversely associated with BC risk [8].

Pathology studies support the influence of obesity on the BC histopathology. It has been reported that among 1,177 women with invasive ductal BC, those in the highest BMI

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quartile developed more malignant tumors in terms of histological grade, mitotic cell count, and tumor size [9], and these obese patients showed increased lymph node involvement and a higher propensity to distant metastases [10, 11]. Obesity has also been linked to BC recurrence and a lower overall survival (OS) in pre- and postmenopausal women with BC [12, 13]. Weight change might influence the risk of BC and its complications, and the degree of weight increase before menopause might enhance the postmenopausal BC risk [14].

At least in postmenopausal women, the link between obesity and BC is related to the hormonal balance, based on high aromatase levels and the release of growth factors and inflammatory cytokines by adipocytes [15, 16]. This interlink is dependent on several molecular pathways, such as the phosphatydilinositol-3-phosphate/mammalian target of rapamycin (PI3K/mTOR) pathway, natively activated as a checkpoint for nutrient/hormonal cell signaling that regulates the proliferation of both adipocytes and mammary epithelial ductal cells [17]. In this regard, genetic defects in obesity-related genes definitely appear linked to BC [18, 19], and drug targeting of molecular pathways that are deregulated in obesity might help to prevent this cancer in obese women. We review both biological and molecular mechanisms linking obesity to BC in women and the relative implications in patient management.

Molecular Mechanisms Linking Obesity With Breast Cancer

The molecular mechanisms include hormones, adipocytokines, inflammatory cytokines, and reactive oxygen species (ROS), as summarized in Figure 1.

Hormonal Status

Estrogens

The higher estrogen levels in postmenopausal women derive from the aromatization of androstenedione and testosterone in the adipose tissue [20, 21]. Aromatase is increased twofold in obese women [22], and its activity is strongly influenced by both tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, which are usually abundant within the adipose tissue [23, 24]. Aromatase is expressed by undifferentiated adipose fibroblasts but not by mature adipocytes, and the ratio of stromal tissue to adipocytes in breast quadrants is higher in BC [25]. Epithelial tumor cells also produce both TNF-α and IL-11 [24] as anti-adipogenic cytokines and prostaglandin E_2 (PGE_2), resulting in higher aromatase production due to the effect on the promoter I.3/II region (PI.3/PII) promoter region of the aromatase gene [26, 27] via protein kinase (PK) A and PKC signaling [28] (Fig. 2). Also, estrogens regulate the G_1/S phase progression by both c-MYC and cyclin D1 (CCND1), enabling the CCNE-cyclin dependent kinase-2 complexes necessary for phosphorylation of the retinoblastoma gene (*RB*), resulting in the activation of E2F transcription factors. This has been supported by antiestrogen treatment of BC cell lines, which downregulates both c-MYC and CCND1, leading to a quiescent state [29]. Finally, estrogens regulate the insulin receptor substrate-1 (IRS-1) in the breast [30], induce free radical-mediated DNA damage, genetic instability, and gene mutations, and inhibit both DNA repair and apoptosis [31, 32].

Insulin Resistance and Hyperinsulinemia

Excess body weight and adiposity are directly correlated with insulin resistance and compensated by an increased secretion of insulin that ultimately primes both the growth and the aggressiveness of postmenopausal BC [33]. Hyperinsulinemia is also responsible for increased levels of insulin-like growth factor (IGF)-I and -II and reduced hepatic expression of IGF-I binding proteins (IGFBP)-1 and -2, leading to higher circulating levels of free IGF-I [34, 35]. IGF-I receptors, overexpressed by BC cells, and a third receptor, in hybrid form (IR-IGFIR), mediate the effects of both insulin and IGF-I [36, 37]. In this regard, it has been shown that IGFBP-1 inhibits the tumor cell growth in mice transplanted with MCF-7 cells [38]; decreased IGFBP-1 levels in obesity suggest the existence of a mechanism enhancing the growth of BC cells.

IGF-I signaling interacts with estrogens to synergistically induce the mitogenic response in breast epithelial cells by c-MYC and CCND1 [29] and primes the canonic mitogenic RAS/MEK/MAPK/ERK1/2 (mitogen-activated protein kinase/extracellular signal-related kinase 1/2) and PI3K/AKT/mTOR (phoshatidylinositol-3 kinase/murine thymoma viral oncogene homolog/mammalian target of rapamycin) pathways [39]. mTOR and ERK activate S6 kinase-1 (S6K1) and the subsequent phosphorylation of S6 ribosomal protein (S6rp), resulting in increased cell proliferation [40]. S6K1 is also responsible for the phosphorylation of estrogen receptors (ERs) and, in turn, both estrogens and ERs increase IGF-IR signaling [40]. High mTOR activity has been definitely correlated with higher risks of disease progression [41], recurrence [42], short disease-free survival (DFS), and a lower response to tamoxifen [43].

Both hyperinsulinemia and hyperglycemia increase the BC risk through the WNT pathway, which induces the translocation of β-catenin to the nuclei via the canonical WNT/β-catenin loop and primes the transcription of target genes, such as *CCND1* and *c-MYC* [44, 45]. Furthermore, variants of the transcription factor 7-like 2 (TCF7L2), which regulates hepatic glucose production as a part of the WNT/β-catenin signaling cascade, are associated with an increased risk of type 2 diabetes and BC [46]. That high glucose levels are able to amplify WNT/β-catenin signaling provides the link between hyperglycemia and cancer.

Adipokines

Adipokines are small peptide hormonal growth factors; they include leptin, adiponectin, and hepatocyte growth factor (HGF). All of them might contribute to BC development [47]. Leptin directly promotes cell proliferation by its receptor and then through either canonical pathways, such as MAPK/ERK1-2, PI3K/AKT/mTOR, Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3), or noncanonical pathways, such as Jun N-terminal kinase (JNK), PKC, and p38 MAPK, nuclear factor-κB (NF-κB), is activated [48]. Thus, NF-κB is translocated to the nucleus for the transcription of *CCND1*, c-*MYC*, *JUN, FOS,* and *BCL2* [49], regulating cell proliferation. Leptin can also induce the direct activation of ERs in MCF-7 cells, even in the absence of its natural ligand estradiol [50], whereas, as an indirect effect, it intensifies the expression of aromatase [51]. In contrast, leptin decreases AMP-activated protein kinase (AMPK) phosphorylation and increases the

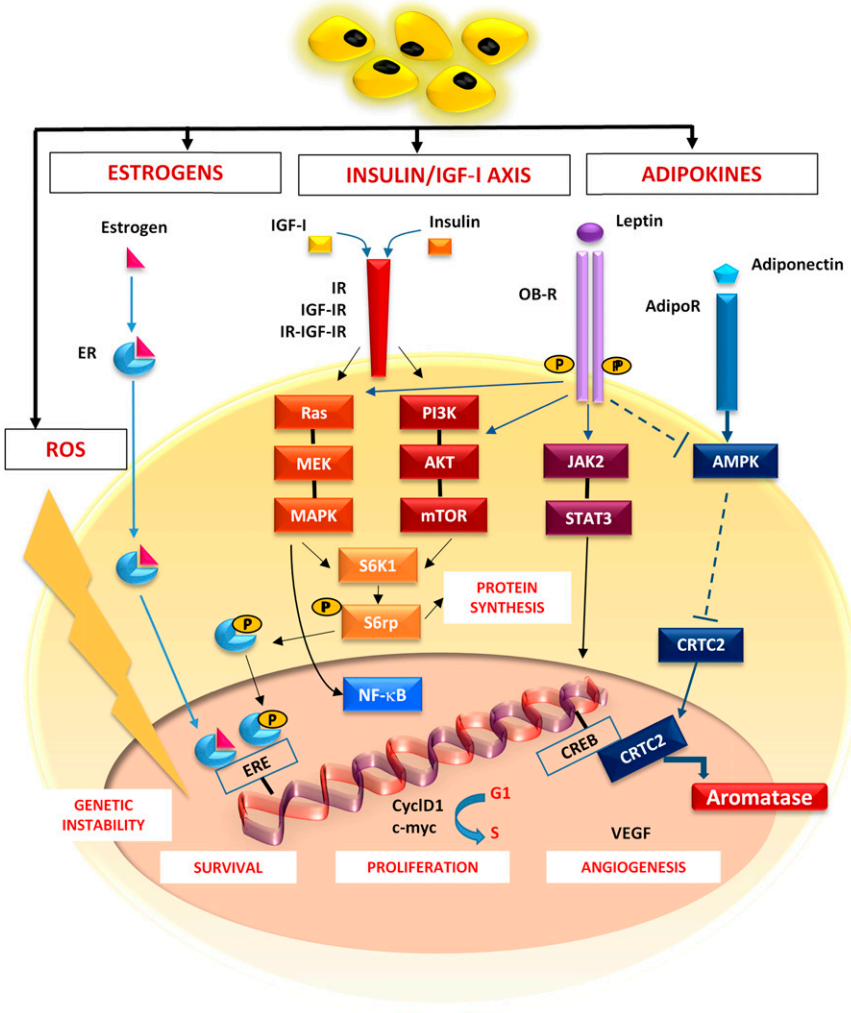

Figure 1. Molecular breast cancer (BC)-related pathways activated in obesity. In obese patients, estrogens, insulin, IGFs, and adipokines activate pathways shown to be deregulated in BC. The estrogen receptor complex migrates to the nucleus and binds the EREs in promoters of target genes that regulate cell survival. Obesity causes increased levels of insulin, IGF-I, and IGF-II that bind the IR, IGF-IR, and a hybrid form (IR-IGF-IR), and thus activate both the RAS/MEK/MAPK and PI3K/Akt/mTOR pathways converging on S6K1. Phosphorylation of S6rp by S6K1 promotes both protein synthesis and cell proliferation. Leptin activates JAK/STAT signaling by its receptor (OB-R). Adiponectin triggers AMPK through its receptor AdipoR. AMPK phosphorylation decreases the nuclear translocation of CRTC2 that binds CREB, thus increasing the aromatase activity. These pathways, converging on NF-κB, lead to the expression of CyclD1 and c-Myc, which promotes cell survival and proliferation, as well as the secretion of VEGF and tumor angiogenesis. ROS contribute to the tumor progression, favoring the genomic instability.

Abbreviations: AdipoR, adiponectin receptor; AMPK, AMP-activated protein kinase; CREB, cAMP response element-binding protein; CRTC2, CREB-regulated transcription coactivator 2; CyclD1, cyclin D1; EREs, estrogen response elements; IGF, insulin-like growth factor; IGF-IR, IGF-I receptor; IR, insulin receptor; JAK2/STAT3, janus kinase 2/signal transducer and activator of transcription 3; NF-κB, nuclear factor κB; PI3K, phosphatydilinositol-3-phosphate; ROS, reactive oxygen species; S6K1, S6 kinase-1; S6rp, S6 ribosomal protein; VEGF, vascular endothelial growth factor.

nuclear translocation of cAMP response element-binding protein (CREB)-regulated transcription coactivator 2 (CRTC2), thus increasing *CYP19A1* expression and aromatase activity [52]. Finally, leptin acts as a proinflammatory protein that promotes monocyte proliferation and macrophage function and reinforces T helper-1 (Th-1) cell proliferation, together with overproduction of TNF-α and other cytokines such as IL-6 and IL-12 [50, 52].

Adiponectin is the most abundant adipokine and enhances cell sensitivity to insulin. This cytokine inhibits the proliferation of a number of cell types and exerts proapoptotic effects, while inhibiting aromatase expression in BC cells [53]. Moreover, adiponectin stimulates intracellular pathways, including AMPK, having an inhibitory effect on the cell cycle via p53 expression. It inhibits protein synthesis through the activation of the tuberous sclerosis complex (TSC)1/TSC2, which is a tumor suppressor complex with a growth inhibitory activity through mTOR suppression. Adiponectin also activates the peroxisome proliferator activated receptor-γ (PPAR-γ) pathway, which, in turn, drives the transcription of other genes that regulate both cell proliferation and differentiation [54]. Therefore, a low adiponectin availability modulates PPAR-γ signaling, causing a subsequent decrease of nuclear levels of the breast cancer gene-1 (*BRCA1*) and alterations of the cellular mechanisms for DNA repair [32, 49].

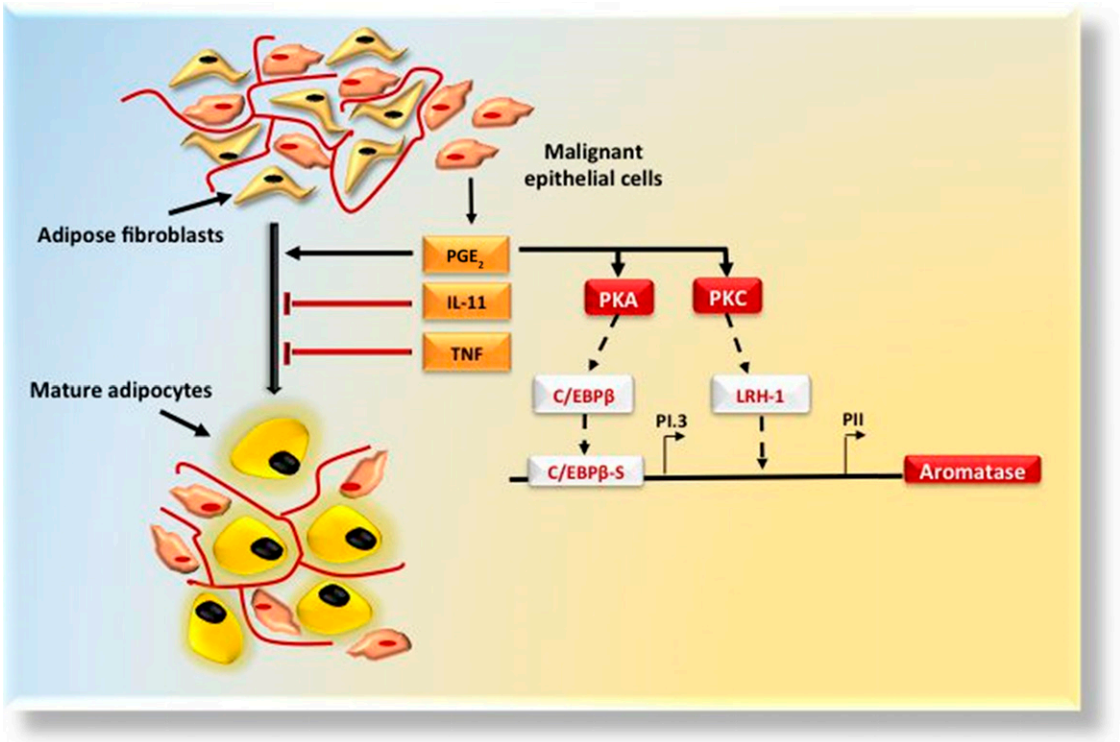

Figure 2. Breast tissue microenvironment: stromal-epithelial interactions. Adipose tissue is a permissive environment for breast cancer (BC). Malignant epithelial cells establish close interactions with adipose fibroblasts that differentiate into mature adipocytes. BC cells produce IL-11, TNF-α, and PGE_2. IL-11 and TNF-α act as antiadipogenic cytokines and PGE_2 reinforces the aromatase expression priming PKA and PKC. PKA activates transcription factor C/EBPβ that binds the key cis-regulatory element C/EBPβS in proximity of the PI.3, and PKC activates the LRH-1 transcription factor whose binding site is located more proximal to PII.

Abbreviations: IL-11, interleukin-11; LRH-1, liver receptor homolog-1; PGE_2, prostaglandin E_2; PI.3, promoter I.3 region; PII, promoter II; PKA, protein kinase A; PKC, protein kinase C; TNF-α, tumor necrosis factor-α.

Inflammation

In obese women, both systemic and local inflammation are usually associated with high levels of inflammatory cytokines (TNF-α, IL-1β, IL-6, and monocyte chemotactic protein 1 [MCP-1]) that activate the transcription of NF-κB in breast tissue [16]. Thus, inflammation of adipose tissue plays a key role in tumor development and progression. Macrophages recruited after adipocyte death [55] form crown-like structures and are detectable both in visceral fat [56] and in mammary glands of obese mice [22], in the presence of high levels of proinflammatory cytokines (IL-1β, IL-6, PGE_2), and with increased *CYP19A1* gene transcription, thereby encoding aromatase [57]. Obesity is also associated with hypertrophy and lipolysis of adipocytes that release free fatty acids and activates NF-κB through Toll-like receptor 4, an immune receptor, ultimately inducing a high bioavailability of TNF-α, IL-6, and other cytokines [55, 58].

A deregulation of PPAR-γ, as a key regulator of adipogenesis in obese patients has been associated with an increased production of the plasminogen-activator inhibitor 1, which is involved in angiogenesis, enhancement of cell adhesion, migration, and inhibition of apoptosis and is suspected of modifying the breast microenvironment and facilitating local cancer development and/or metastasis [59].

Oxidative Stress

Obesity is associated with increased oxidative stress and is characterized by high levels of ROS that variably denature lipids, proteins, and nucleic acids and lead to genetic instability, driving both tumor progression and metastasis by triggering the PI3K/AKT pathway in BC [60]. In addition, ROS and nitric oxide species generated in cancer-associated fibroblasts promote genomic defects in adjacent cancer cells that characterize their typical aggressive behavior [49]. BC cells provoke oxidative stress in adjacent fibroblasts as an engine to fuel their own survival through the production of nutrients from the stromal cells [49].

Gene Polymorphisms Shared by Obesity and BC

Gene polymorphisms and BC risk haven been increasingly described. The most important and penetrant mutations for inherited BC include *BRCA1* and *BRCA2* [61], phosphatase and tensin homologs *(PTEN)* [62], serine-threonine kinase-11 *(STK11/LKB1)* [63], and cadherin 1-type 1 *(CDH1)* genes [64]. However, the association of the defined mutations in these genes with obesity is unclear. Hereditary factors might account for approximately one quarter of interindividual differences that cluster the susceptibility to BC in developed countries, although high-risk mutations appear to be implicated in fewer than 10% of all BC cases [65]. Therefore, a substantial component of the BC risk seems to be the combined effect of a number of low-risk polymorphisms. The role of obesity-related gene polymorphisms in BC development is under investigation, and only a few studies have reported that obesity-related genotypes play a role in determining the

progression of benign breast disease (BBD) to invasive cancer. Because polymorphisms in obesity-related genes can modify the associations between BMI and BC, several polymorphisms in obesity-related genes appear to be significantly associated with a variable risk of BC development. These are summarized in Table 1.

Leptin and Leptin Receptor Single Nucleotide Polymorphisms

Polymorphisms of both leptin (*LEP*) and leptin receptor (*LEPR*) genes have been associated with the development of BC in patients with higher leptin serum levels and leptin overexpression in adipocytes [66]. For example, the *LEPR**6920A allele is associated with decreased risk in women with BBD [67], and the *LEPR* Q223R polymorphism is associated with BC development in East Asians [68] and Tunisians [67], but not in whites or Africans [68]. A significant risk of BC was observed for carriers of the *LEP*-2548A/A genotype ($p = .001$) and carriers of the *LEP*-2548 G/A genotype ($p = .04$) [66, 69] that can be explained by the proximal position of *LEP*-2548G/A to a binding site for the transcriptional factor Sp1 [70]. The occurrence of *LEP*-2548A/A or *LEP*-2548G/A coincides with higher or intermediate leptin mRNA expression, respectively, and cells with *LEP*-2548G/G contain low mRNA leptin levels [70]. In a study relating these associations with the clinical evolution, a shorter DFS was related to higher frequency of *LEP* (−2548) A allele, and OS was reduced in patients carrying the *LEPR* Gln223Arg allele [67]. In contrast, *LEPR* single nucleotide polymorphism (SNP) rs11585329 (622G>T) has recently been associated with improved DFS in women with stage I-II BC [71].

Serum Paraoxonase/Arylesterase 1 SNPs

Paraoxonase/arylesterase 1 (PON1) is an esterase bound to high-density lipoproteins that is disabled in obese subjects [72]. Women carrying at least one copy of the variant *PON1* Gln192Arg allele are at a lower risk of BC compared with women carrying the reference Gln/Gln genotype [67].

TNF-α SNPs

Second-grade obese patients with BC are carriers of allele A of *TNF*-α SNP −308G>A [73], which is responsible for increased serum levels of TNF-α whose contribution to BC development has been definitely shown [74]. Thus, the GA-AA genotypes define the risk factor in HER2+ BC patients with a BMI of 30.0 to >40 kg/m^2 [73].

Fat Mass and Obesity-Associated Gene and Melanocortin Receptor 4 SNPs

Fat mass and obesity-associated gene (*FTO*) is apparently related to obesity and type 2 diabetes [75]. A significant expression of both rs1121980 (T/C) and rs9939609 (A/T) SNPs has been reported in overweight and obese women, even if it has not been associated with BC development [76, 77]. However, Kaklamani et al. found that SNPs in the first intron of *FTO* (rs1477196, rs9939609, rs7206790, and rs8047395) were associated with BC risk [78]. In particular, rs7206790 is predictive for hereditary hormone receptor-positive (HR+) BC in young women.

Mutations in the melanocortin receptor 4 (*MC4R*) gene, such as rs17782313 (T/C), are associated with a monogenic form of extreme, early-onset obesity and are suspected of inducing an increased risk of BC [78]. Women showing the allele combination C/T/C (*FTO* rs1121980/*FTO* rs9939609/MC4R rs17782313) have a 4.59-fold increased risk of developing this type of cancer, independently of age and BMI [77].

Adiponectin (ADIPOQ) and Adiponectin Receptor-1 (ADIPOR1) SNPs

A functional adiponectin (*ADIPOQ*) SNP, rs1501299, is significantly linked to high serum adiponectin levels [79] and BC [80], and rs1501299 (+276C>A) and rs2241766 (+45T>G) are associated with this tumor in whites and African Americans [81] and populations from Kuwait [81] and South India [82]. However, *ADIPOQ* SNP rs1063539 is correlated with improved DFS in women with stage I-II BC [81]; however, a further polymorphism in the *ADIPOR1* SNP rs7539542 is apparently associated with BC risk [83].

β_2-Adrenergic Receptor Gene and β_3-Adrenergic Receptor Gene SNPs

Polymorphisms in the β_2-adrenergic receptor gene (*ADRB2*) are associated with both obesity and type 2 diabetes. Two *ADRB2* variants (rs 1042713G/A and rs1042714G/C) have been related to an increased risk in non-Hispanic women and, in contrast, to a lower risk of BC in Hispanic women [84]. The pro-BC effect of these two variants was higher in women with a BMI ≥25.0 kg/m^2 and in Hispanic women with a history of diabetes [85], suggesting that ethnicity modifies the association between the *ADRB2* G–G haplotype and BC risk and that overweight or obesity might enhance the differing risk between Hispanic and non-Hispanic women.

Glutathione *S*-transferase P1 and M1 SNPs

Obesity decreases the antioxidant defenses by lowering the levels of enzymes, such as catalase, glutathione peroxidase, and glutathione reductase and, concurrently, by altering the activity of cytochrome P-450 [86]. The frequency of heterozygous glutathione *S*-transferase P1 (*GSTP1*) SNP Ile105Val is 1.5 higher in patients with BC, and the frequency of the homozygous form Val/Val is 1.6 higher in BC patients than in control subjects [87]. Finally, an increased risk of BC was found in women with the *GSTM1* null genotype [87].

IGF-I SNPs

Recent studies have shown that IGF-I levels in plasma are correlated with the length of (CA)n repeats in the *IGF-I* gene, although the direction of the correlation remains controversial [88–90]. One study reported a significantly increased BC risk in Chinese women with a higher BMI carrying the (CA)19 allele [91]. However, an increased risk of BC was not found in two other studies [89, 92]. Recently, Pande et al. found an association between the *IGF-I* SNP rs1520220 and poor DFS in women with stage I-II BC [71].

PIK3CA SNPs

The SNP rs2677760 in *PIK3CA* is associated with reduced DFS in women with stage I-II BC [71]. Moreover, a recent meta-analysis showed that *PIK3CA* gene mutations correlate with ER/progesterone receptor (PR) expression ($p < .00001$) and relapse-free survival ($p = .03$), but not OS in unsorted BC patients [92]. It is conceivable that SNPs in *PI3K-AKT-mTOR* genes,

Table 1. Polymorphisms in obesity-related genes

Gene acronym	Gene name	SNP ID/allele	Nucleotide change	Position	Functional effect	Reference
LEP	*Leptin*	rs7799039	−2548G>A	Promoter	Significant risk for BC	66, 69
					Larger tumor size and shorter DSF	66
LEPR	*Leptin receptor*	rs1045895	16734G>A (mRNA 6920G>A)	3′UTR	Decreased risk of BC	67
		rs1137101	177266A>G (mRNA 668A>G	CDS (Gln223Arg)	Increased risk in East Asians and Tunisians	67
		rs11585329	192567G>T	Intron	Shorter OS	71
					Improved DFS in stage I-II BC	
PON1	*Serum paraoxonase/ arylesterase 1*	rs662	21439A>G (mRNA 575A>G)	CDS (Gln192Arg)	Lower risk of BC	67
		rs854560	12801T>N (mRNA163T> A/G)	CDS (Leu55Met)	Increased risk of BC	73
TNF-α	*TNF-α*	rs1800629	−308G>A	Promoter	Increased levels of TNF-α, higher risk of BC	74
					BC risk in patients with BMI >30 kg/m^2, Her2+	73
FTO	*Fat mass and obesity-associated gene (NG_012969.1)*	rs1121980	76393C>T	Intron	Association with overweight and obesity	76, 77
		rs9939609	87653T>A	Intron	BC risk	78
		rs1477196	75384A>G	Intron	Obesity and BC risk	78
		rs9939609	87653T>A	Intron	Predictive of HR+ BC in young women with family history and advanced stages at diagnosis	78
		rs8047395	65649A>G	Intron		78
		rs7206790	65034C>G	Intron		
FTO+ MC4R		fto rs 1121980/fto rs9939609/mc4r rs 17782313	Association of a cluster of SNPs	Intron/Intron/ near the gene	4.59-fold increased risk BC independently of age and BMI	77
MC4R	*Melanocortin receptor 4*	rs17782313	Chromosome 18: 57,851,097T>C	Near the gene	Increased risk of BC	78
ADIPOQ	*Adiponectin*	rs1501299	15661G>T	Intron 3	Increased risk of BC in whites, African Americans, in populations from Kuwait and South India	81, 82
		rs2241766	15430A>C (mRNA +45T>G)	CDS (Gly 15)	Improved DFS in stages I-II of BC	71
		rs1063539	19930G>A/C (mRNA 2899G> A/C)	3′UTR		
ADIPOR1	*Adiponectin receptor-1*	rs7539542	2114G>C (mRNA 727G>C)	3′UTR	BC risk	80
ADRB2	*Beta-2 adrenergic receptor gene*	rs1042713	5285A>G (mRNA 46A>G)	CDS (Gly16Arg)	Increased risk of BC in patients with BMI >25, non-Hispanic women; lower risk in Hispanic women	85
		rs1042714	5318C>G (mRNA 79C>G)	CDS (Gln27Glu)		

(continued)

Table 1. (continued)

Gene acronym	Gene name	SNP ID/allele	Nucleotide change	Position	Functional effect	Reference
GSTP1	*Glutathione S-transferase P1*	rs1695	6624A>G (mRNA 313A>G)	CDS (Ile105Val)	Frequency 1.5–1.6 times higher in BC	87
GSTM1	*Glutathione S-transferase M1*	Null genotype			Increased risk of BC	87
IGF-1	*Insulin-like growth factor-1*	(CA)19 allele			Significantly increased BC risk in Chinese women	85
		rs1520220	82857C>G	Intron	Worse DFS in women with stages I-II of BC	71
PIK3CA	*Phosphatidylinositol-4,5-bisphosphate 3-kinase, sub a*	rs2677760	41954C>T	Intron	Worse DFS in women with stages I-II of BC	71
PR	*Progesterone receptor*	PROGINS A1/A1	Cluster of mutations: 320 bp PV/HS-1 Alu insertion in intron G and two point mutations, V660L in exon 4 and H770H (silent substitution) in exon 5	Intron/exon	Postmenopausal obesity in women with BC	93
		rs10895068	+331G>A (−420G>A)	Promoter	Significantly higher risk of BC (compared with subjects with the GG genotype) through the increased production of the polymorphic variant hPR-B	94
AIBI	*Amplified in breast cancer 1*	AIB1 LG	Longer polyglutamine repeats		Postmenopausal obesity in women with BC	93

Abbreviations: BC, breast cancer; BMI, body mass index; bp, base pair; DFS, disease-free survival; HR+, hormone receptor-positive; OS, overall survival; TNF-α, tumor necrosis factor-α; UTR, untranslated region.

such as *ADIPOQ, IGF1, INS, IRS1, LEP, LEPR, LEPROT, PIK3CA, PTEN, TSC1, TSC2,* and *AKT1*, might simultaneously affect body weight and decrease the responsiveness to BC treatment [71].

PR and Steroid Hormone Receptor Coactivator (AIB1) SNPs

The combined inheritance of the polymorphic variant *PROGINS* A1/A1 (PR SNPs), *AIB1* long polyglutamine genotypes and an early age of menarche (12 years) are risk factors for obesity. The absence of the *PR* polymorphism PROGINS, together with longer polyglutamine repeats in *AIB1*, was associated with postmenopausal obesity in women with BC [93]. Moreover, De Vivo et al. observed a significantly higher risk of BC among carriers of the *PR* +331 A allele compared with subjects with the GG genotype [94]. They also observed a potential interaction between the genotype and BMI among postmenopausal women, with the highest risk for obese women (BMI >30 kg/m^2) with the GA or AA genotype compared with lean women (BMI <25 kg/m^2) with the GG genotype [94]. Their findings suggest that an increased production of polymorphic variant h*PR*-B by the *PR* +331 G/A might predispose women to BC through an increased hPR-B-dependent stimulation of mammary cell growth [94].

Obese Women at Risk of BC

The chronic inflammatory state typical of obesity is due to increased levels of C-reactive protein, inflammatory cytokines, such as TNF-α, IL-6, IL-8, and MCP-1, and leptin [22]. Accumulating evidence has suggested a positive correlation between their high bioavailability and BC incidence. High levels of leptin, in particular, might apparently define a generally increased risk of, and poor prognosis in, BC [95], because postmenopausal patients express high leptin mRNA in adipose tissue and elevated serum levels in association with increased estradiol [96]. Moreover, ER-positive (ER+) tumors have been shown to express high intratumoral levels of leptin, which is specifically involved in cancer growth through an autocrine mechanism [95, 97]. Leptin has been identified as an independent predictive variable of BC pathological tumor size and TNM stage in several studies [52]. In line with these results, clinical studies have recently demonstrated that serum leptin levels correlate with total body aromatase activity in postmenopausal BC patients [98].

Table 2. A potential breast cancer risk profile in obese women

Soluble factors		Level
C-reactive protein		High
Inflammatory cytokines		
TNF-α		High
IL-6		High
IL-8		High
MCP-1		High
Adipokines		
Leptin		High
Adiponectin		Low
Insulin		High
IGF-1		High
Estradiol		High
HGF		High
PAI-1		High
VCAM-1		High
Transcriptomic profile		
Biological process	**Gene**	**Expression**
EMT	*TWIST*	Upregulated
Protein synthesis	*EIF1*	Upregulated
Notch signaling	*ASCL1*	Upregulated
Lipid and cholesterol metabolism	*AKRC1*	Upregulated
	PLIN	Upregulated
	CAV1	Upregulated
	LPL	Upregulated
Inflammation	*IL8*	Upregulated
	IL33	Upregulated
	S100A8	Upregulated
Proliferation	*POLD3*	Upregulated
	G0S2	Upregulated
Adipokines	*ADIPQ*	Upregulated
	LEP	Upregulated
Insulin signaling	*INSR*	Upregulated
Energy production	*ATP9A*	Upregulated
Cell adhesion	*COL5A1*	Downregulated
	MSLN	Downregulated
	MUC1	Downregulated
Suppressor of STAT1 signaling	*PIAS1*	Downregulated
ER signaling	*ESR1*	Downregulated
IGF signaling	*IGF signature*	Activated
SNPs		
Gene name	**SNP allele**	**Effect**
LEP	rs7799039 A	Predisposing
	rs7799039 G	Protective
LEPR	rs1045895	Protective
	rs11585329	Protective
PON1	rs662 A	Protective
	rs854560 A/G	Predisposing
FTO+MC4R	FTO rs 1121980 C	Predisposing
	FTO rs9939609 T	Predisposing
	MC4R rs 17782313 C	Predisposing
SNPs		
Gene name	**SNP allele**	**Effect**
ADIPOQ	rs1501299 T	Predisposing
	rs2241766 C	Predisposing
	rs1063539 A/C	Predisposing
TNFα	rs1800629 A	Predisposing
IGF-1	(ca)19 allele	Predisposing
PR	rs10895068 A	Predisposing

Abbreviations: EMT, epithelial-to-mesenchymal transition; ER, estrogen receptor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL, interleukin; MCP, monocyte chemotactic protein; SNP, single nucleotide polymorphism; STAT1, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α; VACM-1, vascular cell adhesion molecule-1.

The chronic inflammatory state typical of obesity is due to increased levels of C-reactive protein, inflammatory cytokines such as TNF-α, IL-6, IL-8, and MCP-1, and leptin. Accumulating evidence has suggested a positive correlation between their high bioavailability and the BC incidence.

Unlike leptin, adiponectin levels are inversely related to cancer occurrence and stage [49]. Two case-control studies also proved this inverse relation with the risk of BC in pre- and postmenopausal women [79, 99, 100]. Also, serum adiponectin levels negatively correlate with BMI and visceral adiposity, and both insulin and estrogens might suppress its secretion [52, 101]. Adipocytes and stromal cells in adipose tissue are the main sources of another adipokine, namely the HGF. Serum HGF levels correlated positively with the BMI, high stage, ER−, degree of differentiation, and presence of lymph node and distant metastases in patients with locally advanced BC [102]. In a murine model, obesity correlated with the activation, by HGF, of the c-MET axis, involved in the pathogenesis of basal-like BC [103]. Moreover, in a case-cohort study, hyperinsulinemia was an independent risk factor for BC in postmenopausal women, and fasting levels of total IGF-I, free IGF-I, IGFBP-3, and glucose were not associated with the risk of BC [104].

Because several studies showed a specific transcriptomic profile in samples of BC from obese women, the detection of particular signatures in premalignant dysplastic lesions of obese women might serve as a tool for risk prediction, as demonstrated for ERα mutations [105]. Analysis of the transcriptomic profile of pretreatment biopsies from a prospective cohort of 137 ER+ BC patients revealed that 62 genes were significantly overexpressed in obese patients and 50 additional genes were downregulated ($p < .01$) [106]. These genes are involved in several biological processes such as the epithelial-to-mesenchymal transition (EMT), protein synthesis, Notch signaling, inflammation, proliferation, lipid and cholesterol metabolism, adipokines, energy production, cell adhesion, and the suppression of STAT1 signaling (Table 2). In the same study, gene set enrichment analysis revealed an association between obesity and an upregulation of AKT target

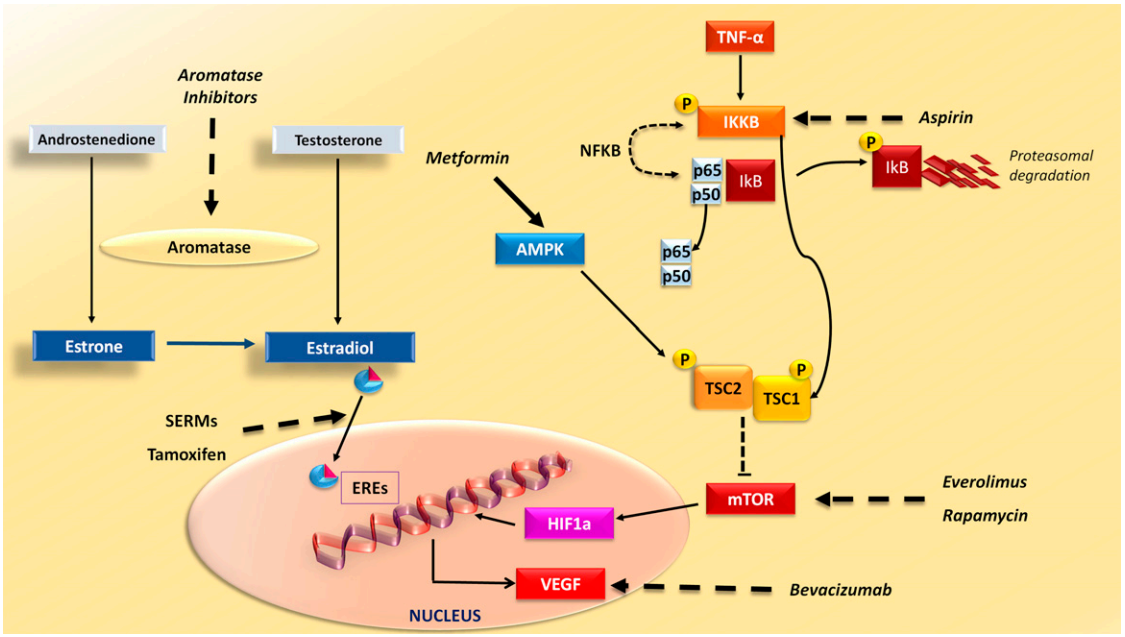

Figure 3. Drugs interfering with breast cancer (BC)- and obesity-related pathways. Drugs commonly used in BC, obesity, and diabetes interfere with intracellular signaling hyperactivation in BC. Aromatase inhibitors inhibit enzyme aromatase in the conversion of androgens, such as testosterone and androstenedione, to estrogens, such as estradiol and estrone. SERMs, such as tamoxifen, bind to estrogen receptors and induce conformational changes that inhibit the expression of estrogen-related genes. In BC cells, high levels of proinflammatory cytokines such as TNF-α, typical in obese patients, activate IKKB that phosphorylates IkB, thus activating NF-κB, composed of its subunits p50 and p65. Furthermore, phosphorylation of TSC1 by IKKB activated by mTOR kinase is involved in cell growth and angiogenesis. Drugs targeting IKKB, such as aspirin, and mTOR inhibitors, such as everolimus and rapamycin, are potentially effective as anti-BC drugs in obese women. Metformin, an antidiabetes drug that can effectively reduce body weight, activates AMPK, which phosphorylates TSC2, having an inhibitory effect on mTOR. Activation of the mTOR pathway leads to the production of VEGF, mediated by transcription factor HIF1α, which could be targeted by bevacizumab.

Abbreviations: AMPK, AMP-activated protein kinase; EREs, estrogen response elements; HIF1α, hypoxia inducible factor-1α, IKKB, IkB kinase β; NF-κB, nuclear factor κB; SERMs, selective estrogen receptor modulators; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

genes involved in glucose metabolism, EMT, and metastasization [106]. The evidence that hyperactivity of the AKT/mTOR pathway in obese mice accelerates mammary tumor growth supports this finding. Microarray data on transgenic mouse tumors identified 1,603 genes with a statistically significantly altered expression, related to 42 biological processes [106]. Statistical evaluation led to the identification of many biological functions concordantly affected by obesity and linked to hallmarks of cancer, in both humans and mice [106]. These processes are primarily associated with metastasis, tumor-promoting inflammation, resistance to cell death, and, above all, a reinforced cell proliferation in ER+ BC.

Furthermore, an obesity-associated BC transcriptome signature has been delineated in a set of 103 tumors, and elevated *IGF* gene scores were observed in the obese tumor group compared with the other tumor group. Also, within the same cohort, an inverse correlation was found between ER gene (*ESR1*) mRNA levels and obesity signature scores [107]. In that study, the investigators also evaluated the overlap between obesity and gene signatures of oncogenic pathways, including IGF-I, PI3K, MAPK, and estrogen (17β-estradiol) and found a significant overlap between underexpressed genes in tumors from obese patients and genes repressed by these pathways, but no overlap was found between overexpressed genes that are high in obesity-associated tumors and the genes activated by these intracellular pathways. They concluded that the obesity signature was distinct from the previously characterized oncogenic pathway signatures [107, 108].

BC development in obese women is also related to a genetic susceptibility, that is specific to each ethnicity, as revealed by the analysis of several gene SNPs (Table 1). In whites, major polymorphisms associated with the BC risk in obese women include *LEP*-2548 AA [66], *PON1* Leu55Met [68], *TNF*-α-308G>A [73], *FTO-MC4R* (*FTO* rs1121980; *FTO* rs9939609; *MC4R* rs17782313) [77], *ADIPOQ* (rs1501299 T; rs2241766 C; rs1063539 A/C) [71, 80, 81], *IGF-I* (CA)19 allele [87], and *PR* (rs10895068 A) [94]. In contrast, other polymorphisms of obesity-related genes exert a protective effect (e.g., *LEPR**6920A, associated with a decreased risk of BC in women with BBD [67]; *LEP*-2548G/G, related to the expression of low leptin mRNA levels [70]; and *LEPR* SNP rs11585329 [622G>T]) and have been associated with improved DFS in women with early-stage BC [71].

Thus, the detection of soluble molecules, expression profiles, and the identification of SNPs could depict a risk signature for BC in obese women, as proposed in Table 2, that could help to plan adequate prevention strategies for obese patients.

Targeting BC and Obesity Interlinked Pathways

The metabolic changes associated with obesity lead to alterations of several pathways targeted by drugs used in BC treatment (Fig. 3). Because estrogens are major inducers of HR+ BC, aromatase inhibitors (AIs) are the standard of care for

Table 3. Ongoing trials evaluating behavioral or medical interventions for BC in obese women

Study	Name	Intervention	Status
NCT01302379	Reach for Health Study: Obesity-related Mechanisms and Mortality in Breast Cancer Survivors	Metformin Lifestyle Diet	Recruiting
NCT02432950	Pancreatic Nutritional Program for Weight Loss in Overweight/Obese Patients With Stage I-III Breast Cancer	Diet	Not yet recruiting
NCT02028221	Phase II Study of Metformin for Reduction of Obesity-Associated Breast Cancer Risk	Metformin	Recruiting
NCT01758146	Impact of Obesity on the Efficacy of Endocrine Therapy With Aromatase Inhibitors	Tamoxifen Letrozole	Recruiting
NCT01793948	Metformin Hydrochloride vs. Placebo in Overweight or Obese Patients at Elevated Risk for Breast Cancer	Metformin	Recruiting
NCT02424292	Evaluation of a Physical Activity Program in Overweight Breast Cancer Patients (I-Move)	Physical activity	Recruiting
NCT02037542	A Study of Lifestyle Intervention in Overweight or Obese Women With Early Stage Breast Cancer	Diet Physical activity	Recruiting
NCT02538484	Impact of Omega 3 Fatty Acid Supplementation on Aromatase in Obese Subjects	Letrozole Fish oil	Recruiting
NCT01627067	Exemestane-RAD001-Metformin	Everolimus Exemestane Metformin	Ongoing, not recruiting
NCT02224807	Effects of Diet and Exercise on Ductal Carcinoma In Situ (DCIS)	Diet Exercise	Recruiting
NCT01784042	Dietary Energy Restriction and Omega-3 Fatty Acids on Mammary Tissue	Diet Omega-3 fish oil	Recruiting

Data from ClinicalTrials.gov (http://www.clinicaltrials.gov).

postmenopausal women, in both the adjuvant and the metastatic setting [109], although anastrozole and letrozole incompletely suppress both estrone and estradiol levels in overweight and obese women [110]. In the ATAC trial (Arimidex, Tamoxifen, Alone or in Combination) comparing anastrozole to tamoxifen in the adjuvant setting, a higher BMI was associated with a higher recurrence in the overall population and in the population receiving anastrozole. The investigators observed that the benefits of anastrozole were lower in women with a higher BMI but that those of tamoxifen were similar, regardless of the BMI [111]. In the ABCSG 12 trial (Austrian Breast and Colorectal Cancer Study Group trial 12), performed in premenopausal women, both overweight and obese patients receiving anastrozole had an increased risk of disease recurrence and death. However, this result was not detected in the group of patients treated with tamoxifen [112]. In contrast, a side effect of all AIs is the weight gain related to the reduction of estrogen levels [113].

Because estrogens are major inducers of HR+ BC, aromatase inhibitors are the standard of care for postmenopausal women, in both the adjuvant and the metastatic setting, although anastrozole and letrozole incompletely suppress both estrone and estradiol levels in overweight and obese women.

Another signaling pathway involved in the BC pathogenesis is the PI3K/AKT/mTOR pathway, which is targeted by mTOR inhibitors such as everolimus. This drug was approved for the treatment of advanced metastatic ER+, HER2− BC, in association with exemestane. Everolimus can overcome the resistance to endocrine therapy, thus prolonging progression-free survival [114]. The results from the GINECO study (Groupe d'Investigateurs Nationaux pour l'Étude des Cancers Ovariens et du sein) have confirmed the importance of this therapeutic approach, demonstrating the improved clinical benefit rate and improved time to progression and OS [115]. Combined therapy against mTOR and IGFI-R is currently being investigated in other clinical trials [116].

In obese patients, activation of the mTOR pathway was related to high levels of cytokines such as TNF-α that, by activating IkB kinase β (IKKβ), lead to the phosphorylation of TSC1, mTOR activation, and production of high levels of vascular endothelial growth factor (VEGF), whose expression was associated with a poor outcome for BC patients [117]. The establishment of mammary tumors in mouse models of genetic and diet-induced obesity revealed higher tumor growth in obese than in lean mice. It is noteworthy that high levels of phosphorylated IKKβ and S6, as indicators of mTOR pathway activation, were detected by immunofluorescence staining in mammary tumors in these mice [118]. However, targeting the IKKβ/mTOR/VEGF pathway with aspirin (IKKβ inhibitor), rapamycin (mTOR inhibitor), or bevacizumab (VEGF antagonist) induced a significant reduction of mammary tumor size only in obese but not in control mice [118]. In a model of ovariectomized obese mouse with orthotopic BC, the normalization of body weight did not affect AKT/mTOR signaling,

which remained continuously activated even after appropriate weight loss. The finding of high levels of phosphorylated-mTOR and phosphorylated-p70S6K in tumor tissue even after calorie restriction suggests a persistent effect of obesity on tumor activity through AKT/mTOR signaling. In this mouse model, everolimus exerted an antiproliferative effect and blocked mTOR activation, thus prompting the speculation that obese patients could benefit from treatment with mTOR inhibitors [119]. Furthermore, the phosphorylation of IRS-1 by S6K was associated with impairment of the activation of PI3K/AKT by insulin, resulting in insulin resistance [120]. Despite the potential role of mTOR inhibitors in disabling this effect, everolimus and rapamycin per se induce metabolic side effects such as hyperglycemia and diabetes, in addition to dyslipidemia [121]. Thus, in obese and diabetic patients, mTOR inhibition primarily worsens insulin resistance [122].

Metformin is an antidiabetic drug that reduces the body weight and reinforces the anticancer properties through a direct or an indirect insulin-dependent mechanism. Through the activation of AMPK, metformin is also able to directly inhibit mTOR and modulate cell growth [123]. This effect is mediated by phosphorylation of TSC2 and raptor, inducing a direct inhibitory action on mTOR kinase [124]. In vitro studies showed the inhibitory effect of metformin in BC cell lines [125], in ER+ cells in particular, and, recently, in BC stem cells [126]. Additional in vivo studies support the antitumor effect of metformin in murine models of ER− BC [127].

Several clinical studies have suggested that metformin reduced the risk and the development of breast and gynecological cancer [128]. In 2009, an epidemiological study revealed that BC patients receiving neoadjuvant treatment with metformin obtained a higher pathology-demonstrated complete response rate (24%) than did the controls (8.0% in the non-metformin group and 16% in the nondiabetic group) [129]. The recent introduction of mTOR inhibitors in BC treatment and the reported evidence of an antitumor effect of metformin support their potential benefit, especially in obese patients, and several ongoing clinical trials are addressing this topic.

Conclusion

BC is a major health problem worldwide, and both overweight and obesity definitely increase the risk for this tumor. Some ongoing studies are evaluating the potential efficacy of behavioral and pharmacological interventions in preventing the incidence and/or recurrence of BC in overweight women (Table 3). Several molecules link obesity with BC and variably affect intracellular pathways such as MAPK, PI3K/AKT/mTOR, and WNT involved in adipogenesis and cancerogenesis. Obese women at risk of BC are characterized by high circulating levels of estrogens and molecules related to inflammation, insulin metabolism, and other intracellular processes. Monitoring the blood levels of these factors and the early detection of intracellular signaling hyperactivation, such as the IGF pathway, along with the identification of polymorphisms of BC and obesity-related genes, will offer a new diagnostic and prognostic tool for the treatment of BC obese patients (Fig. 4), although other genomic studies are needed to identify a clear, obesity-associated BC transcriptional signature. Early recognition of such a signature, together with compliance to a low-calorie diet in high-risk subjects, might be a prevention strategy that is potentially applicable in clinical practice. Obesity is a BC risk factor that can be corrected with both diet and physical exercise, and obesity-associated cancer gene activation might be modulated by small molecules interfering with intracellular pathways that are deregulated in cancer, such as IGFR, PI3K, and mTOR inhibitors. Because the hyperactivity of these oncogenic pathways is related to a worse prognosis of BC in obese patients, these drugs could potentially be effective in this patient setting, alone or in association with conventional treatments. However, in vivo studies are needed to prove that these compounds are really useful in counteracting tumor growth in obese patients and to ascertain whether a lifestyle change might have a true impact on genetic tumor features or could prevent oncogenesis. Thus, the steps that might affect the growing global problem of obesity-related cancer could include the following points: (a) educational nutrition programs for children and young people, (b) information on healthy lifestyles, (c) early recognition of clinical conditions increasing the risk of cancer in general practice outpatients, and (d) application of genomic tools to identify risk signatures for prevention of BC in obese women.

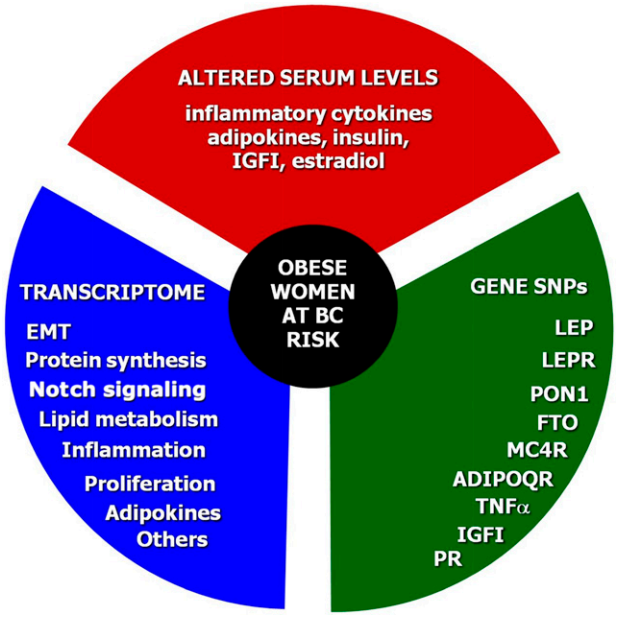

Figure 4. Arbitrary BC risk grading in obese women. Considering the molecular interlinks between obesity and BC, clinical assessment of the BC risk in obese women should include three steps. The first step is to measure the serum levels of inflammatory cytokines, adipokines, insulin, IGFI, and estradiol. The second step should include the assessment of both obesity and BC-related genetic derangements such as SNPs of *LEP, LEPR, PON1, FTO, MC4R, ADIPOQ, TNFα, IGFI,* and *PR*. In the case of premalignant or suspicious lesions, the third step would include the analysis of the transcriptomic profile related to biological processes such as EMT, protein synthesis, Notch signaling, the lipid and cholesterol metabolism, inflammation, proliferation, adipokines, cell adhesion, estrogen receptor signaling, and others.

Abbreviations: EMT, epithelial-to-mesenchymal transition; IGFI, insulin-like growth factor I; SNPs, single nucleotide polymorphisms.

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Disclosures

The authors indicated no financial relationships.

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