The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression

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Abstract

Epidemiological studies have related hyperinsulinemia and type 2 diabetes to an increased breast cancer risk, an aggressive and metastatic phenotype, and a poor prognosis. Furthermore, diabetic retinopathy arises from pathological angiogenesis, which is also essential for breast cancer growth and metastasis. Insulin stimulates the proliferation of some human breast cancer cell lines *in vitro* by mechanisms that use both the phosphatidylinositol-3 kinase and the mitogen-activated protein kinase/Akt signaling pathways; it is also a cell survival (anti-apoptotic) agent and enhances tumor cell migration and invasive capacity. Hyperinsulinemia affects breast cancer cells via the endocrine system, but experimental studies suggest the importance of paracrine mechanisms operating by the effects of insulin on the secretion of adipokines from tumor-associated adipose tissue. In such a system, one adipokine, leptin, has stimulatory paracrine effects on breast cancer cell proliferation and survival, while a second, adiponectin, is inhibitory. Leptin, vascular endothelial growth factor, another insulin-regulated adipokine, and insulin itself also stimulate angiogenesis. Insulin has complex interactions with estrogens: it induces adipose stromal cell aromatase and tumor cell sex steroid hormone receptor expression and suppresses sex hormone-binding globulin, which may enhance estrogen synthesis and bioactivity with consequent promotion of estrogen-dependent breast cancer. All these actions influence the later steps in breast cancer development but genetic studies are also revealing connections between gene abnormalities related to type 2 diabetes and the initiation stage of breast carcinogenesis. Understanding the various mechanisms by which insulin participates in breast cancer cell biology provides opportunities for novel approaches to treatment.

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Introduction

Breast cancer is the most commonly occurring cancer in American women, with the exception of non-melanomatous skin cancer, and is second only to lung cancer as the most common cause of cancer-related deaths ([American Cancer Society 2011](#)). There is also a high prevalence of diabetes in the United States; it was estimated that in 2005–2006, 7.7% of the population aged 20 years or older had been diagnosed as being diabetic and another 5.1% had unrecognized diabetes ([Cowie *et al.* 2006](#)).

There are broadly two forms of diabetes mellitus: in type 1, diabetes is diagnosed most often in childhood and adolescence and results from an absolute

deficiency of insulin that arises from autoimmune destruction of the pancreatic β -cells. Type 2 diabetes, which constitutes ~95% of all cases ([American Diabetic Association 2012](#)), develops most often in later life but, together with obesity, is exhibiting an increasing prevalence in children and young adults. An autoimmune-related loss of β -cells is not involved, but both a reduction in insulin secretion and a metabolic resistance to insulin action are present and responsible for decreased glucose peripheral uptake and increased hepatic glucose production. In the preclinical and early clinical stages of type 2 diabetes, a high plasma insulin concentration features prominently among the biochemical abnormalities.

However, despite the presence of hyperinsulinemia, longitudinal studies demonstrated that compromised β -cell function is present in prediabetics years before the onset of type 2 diabetes (Festa *et al.* 2006).

Obesity is also a risk factor for postmenopausal breast cancer (Rose *et al.* 2004) and is also causally associated with insulin resistance and an increased risk for type 2 diabetes. In studies that included analyses with statistical adjustments for confounding, hyperinsulinemia and type 2 diabetes were shown to be independent risk factors for postmenopausal breast cancer (reviewed by Vona-Davis & Rose (2012)). A meta-analysis of 20 studies from nine different countries showed a 20% increase in breast cancer risk in women with diabetes compared with nondiabetics, with adjustment for the BMI having no significant effect on this result: the relationship was confined to women who were beyond the menopause (Larsson *et al.* 2007). A large prospective study by Michels *et al.* (2003) confirmed the positive relationship in postmenopausal women but actually found a trend for a negative relationship for premenopausal women.

Our present purpose is not to examine the epidemiological evidence that type 2 diabetes is a risk factor for breast cancer and causally related to a poor prognosis, as this has been the subject of a number of recently published reviews (for example, Wolf *et al.* (2005), Xue & Michels (2007), Vigneri *et al.* (2009), Peairs *et al.* (2011) and Johnson *et al.* (2012)). Here, the focus is specifically on insulin as a hormonal factor in breast cancer biology and its actions on tumor cell growth and invasion and tumor-related angiogenesis. The mechanisms involved are explored, with a discussion on insulin as a direct breast cancer cell and vascular endothelial cell mitogen and anti-apoptotic agent and as a potential endocrine regulator of three breast cancer-associated adipokines that are concerned with paracrine and autocrine growth control. In addition, clinical aspects of insulin-estrogen interactions are discussed.

Hyperinsulinemia

Insulin synthesis, secretion, and response to peripheral resistance

The primary translation product of the insulin gene is preproinsulin, a 110 amino acid peptide that is processed in the pancreatic β -cells to yield proinsulin: this, in turn, loses a connecting peptide (C-peptide) to produce insulin. Plasma proinsulin was shown by Zethelius *et al.* (2003) to be a biomarker of type 2

diabetes risk and is a highly specific marker for insulin resistance (Pfutzner *et al.* 2004).

Insulin pathophysiology has two components: one is functional and arises from abnormal insulin secretion kinetics and reduced insulin sensitivity and the other is manifestly pathological in the sense that there is a quantifiable diminution in pancreatic β -cell mass with heightened apoptotic activity (Guillausseau *et al.* 2008). A genetic component to type 2 diabetes risk is now well recognized (Zeggini & McCarthy 2007). In individuals with a genetically determined risk for type 2 diabetes, loss of β -cell activity results in the failure of insulin secretion to compensate for tissue resistance and in consequence there is worsening hyperglycemia. However, in situations where β -cell function is maintained, there is sustained hyperinsulinemia, but the plasma glucose concentration remains normal. So, for example, in obesity, not complicated by type 2 diabetes insulin resistance is compensated for by an increase in β -cell mass but the elevated insulin production may result in hyperinsulinemia with its potentially adverse effect on breast cancer risk. For those who do develop diabetes, hyperinsulinemia may predate clinical disease by at least 10–15 years (Lundgren *et al.* 1990).

Breast cancer

There is a wide range of biological differences between breast cancers arising in premenopausal and postmenopausal women, which are expressed clinically in the relatively high prevalence of estrogen receptor (ER)-negative, hormone-independent, aggressive tumors in premenopausal breast cancer. Upper body obesity is a risk factor for postmenopausal breast cancer, whereas in premenopausal women, adiposity does not usually enhance risk and may actually exert a protective effect (van den Brandt *et al.* 2000, Lahmann *et al.* 2004).

Gunter *et al.* (2009) compared the fasting serum insulin concentrations on entry into a prospective study of 835 nondiabetic postmenopausal women who subsequently developed breast cancer and 816 women who remained cancer free. They found a positive association with breast cancer risk: the hazard ratio for the highest compared with the lowest quartile of insulin values was 1.46 (95% confidence interval: 1.00–2.13); $P_{\text{trend}}=0.02$. In women participating in the Women's Health Initiative Study, the determination of fasting insulin concentrations was repeated at intervals during the follow-up period (Kabat *et al.* 2009). Women with serum insulin levels in the upper tertile were more than twice as likely to develop breast

cancer compared with those in the lowest tertile; moreover, the relationship between the fasting serum insulin concentration and breast cancer risk held true for lean postmenopausal women, indicating a mechanism independent of the effects of obesity. The serum glucose and insulin levels were also used in this study to determine the homeostasis model assessment (HOMA)-insulin resistance index. This showed a positive association between the HOMA-insulin resistance and breast cancer risk, as did a prospective study performed in Italy by [Sieri *et al.* \(2012\)](#). High fasting serum insulin concentrations and indices of insulin resistance were also demonstrated by [Goodwin *et al.* \(2002, 2009\)](#) to be associated with a poor prognosis in women with early breast cancer.

In summary, epidemiological studies have demonstrated a positive association between hyperinsulinemia and breast cancer risk in nondiabetic postmenopausal women which, with the usual concern that there may be some residual confounding in the statistical evaluation, appears to be independent of the degree of adiposity. Before the menopause, the situation is much more complex. It was discussed in detail recently by [Vona-Davis & Rose \(2012\)](#), but the overall conclusion from the published results of both prospective and case–control studies is that there is no consistent association between premenopausal breast cancer and the serum insulin.

Insulin: a direct breast cancer cell mitogen and cell survival factor

Insulin, its receptor, and signaling pathways

Although insulin is involved primarily in the regulation of carbohydrate, lipid, and protein metabolism, it also has a significant role as a growth factor: it stimulates cell mitosis and migration and inhibits apoptosis, effects that may actually be increased under conditions of insulin resistance and consequent impairment of insulin-regulated metabolic pathways. In general, the metabolic effects of insulin such as glucose transport are mediated by way of the phosphatidylinositol 3-kinase (PI3K) pathway, whereas the mitogenic effects of insulin involve the activation of Ras and the mitogen-activated protein kinase (MAPK) pathway ([Fig. 1](#)). When insulin resistance with hyperinsulinemia is present, the capacity for stimulation of the PI3K pathway by insulin is lost, but there is enhanced MAPK activation and an increase in insulin-induced prenylation of Ras protein ([Gallagher & LeRoith 2010](#), [Draznin 2011](#)). As will be seen later, this distinction between the two signaling pathways is not absolute and

is not a consistent feature of the mitogenic effects of insulin on cancer cells.

Specific insulin receptors (IRs) are present at the surface of most cells, although the levels are particularly high on targets for insulin action. The IR is a member of the tyrosine kinase family of receptors and is a heterotetramer composed of two extracellular α -subunits and two transmembrane β -subunits that are linked by disulfide bonds. Insulin binds to the α -subunits to activate the tyrosine kinase of the β -subunits. Amplification of the IR tyrosine kinase results in the rapid phosphorylation of several proteins, including Shc adaptor protein 1 and members of the IR substrate (IRS) family. One of these, IRS1, acts as an intermediate downstream docking protein and forms a scaffold for the downstream partitioning of the PI3K and MAPK signaling pathways ([Fig. 1](#)). In this manner, the IRS adaptor protein coordinates signaling mediated by hormones and growth factors, including insulin and insulin-like growth factor (IGF1), as well as the adipokine leptin (reviewed by [Fruhbeck \(2006\)](#)), downstream of the individual activated cell surface receptors. Binding of IRS to IR activates the PI3K pathway, with further activation of Akt, and binding of Shc adaptor protein 1 to IR activates the Ras/MAPK pathway ([Fig. 1](#)). The expression of IRS1 is increased in breast cancer tissues, with particularly high levels in well-differentiated compared with poorly differentiated tumors ([Sisci *et al.* 2007](#)).

Breast cancer cell IR and mitosis

The significance of the IR to cancer cell biology and the implications for cancer treatment and control were the subject of a recent in-depth review by [Belfiore & Malaguarnera \(2011\)](#) and will only be discussed briefly here. In a study of 191 patients, higher primary breast cancer IR levels were found to be associated with biomarkers of a good prognosis, including low histological grade, progesterone receptor (PR) expression, and an absence of regional lymph node involvement, and with prolonged periods of distant disease-free survival ([Mulligan *et al.* 2007](#)). The IR is distinct from the receptor for IGF1; cross-binding of the two ligands does occur but with low affinities, and so at physiological concentrations, insulin and IGF1 activate separate biological responses. Nevertheless, the receptors do share the same adaptor molecules that provide entry to the same signaling pathways and this commonality may limit the efficacy of small-molecule IGF1 receptor inhibitors as chemotherapeutic agents ([Buck *et al.* 2010](#)).

Figure 1 The Ras/MAPK and PI3K/Akt/mTOR signaling pathways, NF- κ B nuclear translocation, and breast cancer cell proliferation and survival (anti-apoptosis). I κ B kinase phosphorylates NF- κ B-bound I κ B proteins and released NF- κ B dimers translocate to the nucleus. PI3K activation of Akt stimulates NF- κ B activity, which also occurs via Ras/Raf. IRS1, insulin receptor substrate-1; MEK, mitogen-activated protein kinase kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin; NF- κ B, nuclear factor κ -light chain enhancer of activated B cells. Full colour version of this figure available via <http://dx.doi.org/10.1530/ERC-12-0203>.

Although proinsulin is present in the plasma and occurs at high levels in type 2 diabetes, it was regarded as having only weak affinity for the IR, little biological activity, and of no relevance to breast cancer biology. However, the situation has changed with the demonstration that IR actually occurs in two splice variants, isoforms IR-A and IR-B, and that IR-A is particularly active in mitotic signaling (reviewed by Belfiore *et al.* (2009)). Sciacca *et al.* (1999) found that IR-A was dominant in breast cancer tissues and that its activation

by either insulin or IGF2 stimulated mitosis. Later, this same group showed that in the MDA-MB-157 human breast cancer cell line, proinsulin binds with high affinity to IR-A and stimulates phosphorylation of the receptor (Malaguarnera *et al.* 2012).

Numerous cell culture experiments have been performed to examine the effect of insulin on breast cancer cell growth using the assessment of [3 H]thymidine incorporation or change in cell number. Although only a few cell lines were used, these showed

consistently that insulin added to serum-free medium promoted growth of the ER-positive MCF-7, T47D, and ZR-75-1 human breast cancer cell lines (Table 1). Ogasawara & Sirbasku (1988) compared the effects of insulin and IGF1 on MCF-7 cell proliferation and found that insulin and IGF1 were mitogenic, although IGF1 was more potent. The T47D cell line was also stimulated by both insulin and IGF1, but only at concentrations that were 15–20 times those required for the MCF-7 cells. Milazzo *et al.* (1992) showed that MCF-7 and ZR-75-1 breast cancer cells had IR levels that were five- and three-fold of those present in an untransformed human breast epithelial cell line or T47D breast cancer cells; in all cases, insulin stimulated IR tyrosine kinase activity and thymidine incorporation.

There have been several investigations directed at understanding the influence of insulin on the PI3K and MAPK signaling pathways and mitosis in breast cancer cell lines *in vitro* (Table 2) and mammary tumor development *in vivo*. Chappell *et al.* (2001) showed that the stimulation of MCF-7 cell proliferation by insulin required activation of PI3K and that this response was accompanied by increased expression of cyclin D1, a PI3K-regulated protein involved in cell cycle progression. Novosyadlyy *et al.* (2010) reproduced the relationship between hyperinsulinemia and breast cancer development using a nonobese but insulin-resistant and hyperinsulinemic, transgenic MKR mouse model. They bred double transgenic PyVmT/MKR female mice that developed mammary ductal hyperplasia and, later, IR-expressing

carcinomas. Signaling by the PI3K, but not the MAPK, pathway was increased in the mammary tissues, and tumorigenesis was suppressed by treatment with a small-molecule tyrosine kinase inhibitor that blocked signaling via the IR.

In contrast, the growth response to insulin of other breast cancer cell phenotypes appears to involve exclusively the MAPK signaling pathway. The ZR-75-1 cell line does express PI3K protein, which is functional as demonstrated by its response to EGF, but in experiments performed by Gliozzo *et al.* (1998), insulin-induced mitosis was not associated with a change in PI3K activity nor blocked by a PI3K inhibitor; it was, however, accompanied by an increase in Shc tyrosine phosphorylation and MAPK activity and suppressed in the presence of an inhibitor of MAPK.

The effects of insulin on ER-negative human breast cancer cell lines are quite complex and their interpretation is limited by the number of cell lines. Gliozzo *et al.* (1998) reported that cultured MDA-MB-157 cells, which do not express ER (Sawatsri *et al.* (2001), showed a strong mitogenic response to exogenous insulin. This stimulation was suppressed by a pharmacological inhibitor of MAPK kinase, which phosphorylates and activates MAPK (Fig. 1), whereas inhibition of PI3K produced only a ‘blunting’ of the growth response, results consistent with a critical role for the Ras/MAPK pathway. This cell line was also used by Malaguarnera *et al.* (2012) to demonstrate that proinsulin and insulin have similar stimulatory effects on MAPK activation and breast cancer cell proliferation and migration.

Table 1 The influence of insulin on the growth of human breast cancer cell lines *in vitro* and their estrogen receptor (ER) status

Cell lines (ER status)	Insulin growth stimulation		Reference
	[³ H]thymidine	Cell number	
MCF-7 (ER+)	+	Not done	Osborne <i>et al.</i> (1978)
	Not done	+	Ogasawara & Sirbasku (1988)
	+	Not done	Milazzo <i>et al.</i> (1992)
T47D (ER+)	Not done	+	Sepp-Lorenzino <i>et al.</i> (1994)
	+	Not done	Ogasawara & Sirbasku (1988)
	+	Not done	Milazzo <i>et al.</i> (1992)
ZR-75-1 (ER+)	+	Not done	Belfiore <i>et al.</i> (1996)
	Not done	+	Osborne <i>et al.</i> (1978)
	+	Not done	Ogasawara & Sirbasku (1988)
MDA-MB-231 (ER–)	Not done	+	Milazzo <i>et al.</i> (1992)
	±	Not done	Gliozzo <i>et al.</i> (1998)
	–	–	Osborne <i>et al.</i> (1978)
MDA-MB-468 (ER–)	±	Not done	Costantino <i>et al.</i> (1993)
	Not done	±	Belfiore <i>et al.</i> (1996)
	Not done	+	Sepp-Lorenzino <i>et al.</i> (1994)
MDA-MB-157 (ER–)	Not done	+	Gliozzo <i>et al.</i> (1998)

±, little or no stimulation.

Table 2 Insulin-induced responses of the PI3K and MAPK signaling pathways in breast cancer cell lines

Cell line	ER status	Insulin growth	Activation by insulin			
			IR	IRS1	PI3K	MAPK/rasGTP ^a
MCF-7	Positive	Yes (1, 2, 4, 5)	Yes (2)	Yes (4)	Yes (3, 5)	rasGTP yes (4)
ZR-75-1	Positive	Yes (1–3)	No (3)	No (3)	No (3)	MAPK yes (3)
MDA-MB-157	Negative	Yes (3)	Yes (3)	NA	Yes (3)	MAPK yes (3)
MDA-MB-468	Negative	No (4)	Yes (4)	Yes (4)	Yes (4)	rasGTP no (4)
MDA-MB-231	Negative	No (1, 6)	Low (6)	NA	NA	NA

The numbers in parentheses indicate the relevant references as follows: (1) [Osborne *et al.* \(1978\)](#); (2) [Milazzo *et al.* \(1992\)](#); (3) [Gliozzo *et al.* \(1998\)](#); (4) [Sepp-Lorenzino *et al.* \(1994\)](#); (5) [Chappell *et al.* \(2001\)](#); (6) [Belfiore *et al.* \(1996\)](#).

^aMAPK-stimulated activity; rasGTP increased; NA, not available.

The effects of insulin have also been examined in two other ER-negative human breast cancer cell lines, but in contrast to MDA-MB-157 cells, neither showed a significant mitogenic response ([Table 2](#)). [Sepp-Lorenzino *et al.* \(1994\)](#) performed insulin growth experiments with the MDA-MB-468 cell line and found that although the IR was autophosphorylated and there was an activation of IRS and PI3K, these changes were not accompanied by a proliferative response; significantly, there was no activation of Ras by insulin, although epidermal growth factor, which does stimulate MDA-MB-468 cell growth, did produce an increase in rasGTP. The MDA-MB-231 human breast cancer cell line possesses IR at levels similar to those present in some insulin-responsive breast cancer cell lines, but it exhibited minimal or no stimulation of thymidine incorporation or proliferation *in vitro* when cultured in the presence of added insulin ([Table 1](#)), a lack of response that [Costantino *et al.* \(1993\)](#) showed was associated with low IR tyrosine kinase activity.

The failure of insulin to induce a tyrosine kinase response may be associated with a high level of membrane glycoprotein PC-1 expression in MDA-MB-231 cells compared with other cell lines, including the ER-negative MDA-MB-157 cell line that does undergo an insulin-stimulated growth response ([Belfiore *et al.* 1996](#)). The expression of PC-1 was low in MCF-7 cells, but [Belfiore *et al.*](#) prepared clones with different levels of the protein and showed that the PC-1 activity was inversely correlated with IR autophosphorylation. These observations are of particular interest because PC-1, a phosphodiesterase present at particularly high levels in liver, skeletal muscle, and adipose tissue, interacts with the IR to inhibit insulin signaling; its overexpression is related to insulin resistance ([Goldfine *et al.* 2008](#)), and its suppression results in improved insulin sensitivity ([Zhou *et al.* 2009](#)).

Anti-apoptosis

Apoptosis, programmed cell death, is mediated by a series of protein factors that include the pro-apoptotic members of the Bcl-2 family and the caspase group of cysteine proteases. Bcl-2 itself, however, is one of the proteins that inhibit apoptosis. Upregulation of the Bcl-2 family member Bax results in increased activity of the caspases and enhanced apoptotic activity; inhibition of one of these enzymes, caspase-3, is part of the mechanism by which insulin exerts an anti-apoptotic function. In addition to Bax, there are 11 other proteins with varying degrees of structural similarity to Bcl-2; one, Bad, is also downregulated by insulin with a resulting reduction in programmed cell death. The signaling of anti-apoptotic activity by insulin involves both the PI3K/Akt and MAPK pathways ([Desbois-Mouthon *et al.* 2000](#), [Park *et al.* 2000](#)).

Insulin can impede the process of apoptosis in DNA-damaged untransformed breast epithelial cells ([Merlo *et al.* 1995](#)); a similar effect in transformed cells would serve to reinforce the increase in breast cancer cell number produced by enhanced mitotic activity. [Teng *et al.* \(2011\)](#) found that the long-acting insulin analog glargine produced a partial suppression of apoptosis in the MCF-7 breast cancer cell line, which was accompanied by downregulation of Bax and upregulation of Bcl-2 protein, and a similar anti-apoptotic effect of insulin, involving the PI3K pathway was observed in an endometrial cell line ([Wang *et al.* 2012](#)).

Adipokines

Adipokines are proteins, mostly growth factors and cytokines, that are synthesized in adipose tissue including the fat cells or adipocytes, the stromal cell proadipocytes, and the macrophages that infiltrate the adipose tissue mass. There are a number of adipokines that exert positive or negative effects on breast cancer cell growth and invasion; here, we focus

on three: leptin, adiponectin, and vascular endothelial cell growth factor (VEGF). Sites of adipokine secretion include the general body adipose tissue mass, from which their entry into the systemic circulation provides for their endocrine activity; the adipocytes of the tumor capsule and the breast cancer cells themselves are sources of adipokines acting as paracrine and autocrine factors respectively. Figures 2 and 3 summarize these relationships and also the functional interactions that occur when they are coupled with the endocrine activity of insulin secreted from the β -cells of the pancreatic islets.

Leptin

Leptin is a pleiotropic protein secreted by both preadipocytes and adipocytes and activates both the Ras/MAPK and PI3K/Akt signaling pathways (Fruhbeck 2006). Plasma leptin concentrations are positively correlated with the BMI and are abnormally high in obese women (Rose *et al.* 2004). However,

although obesity is a recognized risk factor for postmenopausal breast cancer and an association of hyperleptinemia with increased breast cancer risk was reported, later studies of plasma leptin levels in breast cancer have produced conflicting results (Vona-Davis & Rose 2007). One possible explanation is that a paracrine mechanism pre-empts the endocrine role of leptin-mediated stimulation of breast cancer development and progression; higher levels of leptin expression have been demonstrated in the adipocytes of the stromal capsule, which surrounds a breast tumor mass and this contributes to the cancer cell microenvironment (Celis *et al.* 2005).

Plasma leptin levels are elevated in type 2 diabetes and exhibit a positive correlation with the degree of insulin resistance that is independent of the BMI and body fat mass (Fischer *et al.* 2002, Wauters *et al.* 2003). Insulin regulates leptin availability by stimulating both new leptin synthesis and the release of leptin from pre-existing intracellular pools; this elevation in leptin levels involves interaction between insulin and glucocorticoids and is dependent on the

Figure 2 The paracrine stimulation of breast cancer cell growth and invasion by leptin and inhibition by adiponectin and their enhanced activities by hyperinsulinemia. In the Figures, increases/stimulations are indicated by (open triangle) and decreases/inhibitions by (open down triangle). Full colour version of this figure available via <http://dx.doi.org/10.1530/ERC-12-0203>.

growth. Additionally, hyperleptinemia enhances aromatase activity resulting in increased estrogen synthesis, favoring cross talk between the leptin receptor and the ER α via PI3K/MAPK and JNK signaling pathways (Cirillo *et al.* 2008).

Leptin supports the angiogenic component of tumor development; like insulin, it stimulates vascular endothelial cell proliferation and capillary tube formation *in vitro* and angiogenesis in animal models (reviewed by Vona-Davis & Rose (2009)) and may do so through an adipocyte–endothelial cell paracrine loop (Fig. 3). It should be noted that the paracrine effects are still considered speculative as the data for *in vivo* is different from cell culture. For example, in the fatless A-Zip/F1 mouse, adipose tissue and adipokines such as leptin, are unavailable for tumor development making insulin resistance and inflammation the main mechanisms accounting for tumor growth and progression (Hursting *et al.* 2007).

Although leptin is produced primarily by adipocytes, it can also be secreted by fibroblasts, the principle component of stroma surrounding the tumor. The cancer-associated fibroblasts secrete leptin, which acts in a paracrine fashion to bind its receptor on mutant ER-expressing tumor cells creating an environment of cross talk between stroma and tumor (Barone *et al.* 2012).

Adiponectin

Adiponectin has activities under both physiological and pathological conditions that are largely in opposition to those of leptin. Plasma adiponectin concentrations show an inverse correlation with the BMI and are subnormal in obese women. Both the adiponectin protein levels in plasma (Weyer *et al.* 2001, Haluzik *et al.* 2004) and expression of the mRNA in adipose tissue (Statnick *et al.* 2000) are reduced in type 2 diabetes, changes that are a function of insulin resistance rather than of any coexisting adiposity. Hypoadiponectinemia has been associated with a BMI-independent increase in postmenopausal breast cancer risk (Mantzoros *et al.* 2004, Tian *et al.* 2007, Tworoger *et al.* 2007), and with high histological grade and advanced stage at the time of diagnosis (Miyoshi *et al.* 2003, Hou *et al.* 2007).

Studies showing an association of hypoadiponectinemia and breast cancer risk with hyperinsulinemia and insulin resistance are lacking. Duggan *et al.* (2011) performed a prospective investigation of the fasting serum adiponectin and insulin concentrations and the HOMA-insulin resistance index in stage I and II breast cancer patients and found that higher than median adiponectin concentrations were associated with a decreased risk of breast cancer mortality; the HOMA-insulin resistance values were positively correlated with both the breast cancer-specific and the all-cause mortality, as were, to a lesser degree, the fasting serum insulin levels.

There is experimental evidence that the level of adiponectin in the microenvironment influences the behavior of breast cancer cells. In cell culture experiments, exogenous adiponectin suppressed the proliferation and promoted apoptosis of both ER-positive (Dieudonne *et al.* 2006) and ER-negative (Dos Santos *et al.* 2008) breast cancer cells. Adiponectin also inhibited the invasive capacity of the highly metastatic ER-negative MDA-MB-231 breast cancer cell line *in vitro* and its metastasis *in vivo* (Wang *et al.* 2006). Similar to leptin, the paracrine actions of adiponectin remain speculative *in vivo* and do not support a role for adipose tissue and

adiponectin in the enhancement of mouse mammary tumor development (Hursting *et al.* 2007).

One mechanism by which adiponectin levels are reduced in the tumor cell microenvironment is suggested by the demonstration that prolonged exposure of cultured adipocytes to insulin induces a dose-dependent, reversible, suppression of adiponectin expression (Fasshauer *et al.* 2002). This action of insulin observed *in vitro* may be a contributing factor to the development of mammary cancers in the nonobese diabetic mice with insulin resistance and hyperinsulinemia described by Novosyadlyy *et al.* (2010).

The postulated insulin-mediated cooperative paracrine-positive effects of increased leptin and negative effects of decreased adiponectin expression on breast cancer cell growth and invasion are summarized in Fig. 2.

Vascular endothelial growth factor

VEGF exerts its effects specifically on vascular endothelial cells for which it is a potent mitogen and anti-apoptotic factor and promoter of capillary tube formation (Veikkola & Alitalo 1999, Ferrara 2001). It is synthesized in the preadipocytes and adipocytes where it is essential for vascularization and expansion of adipose tissue. In humans, the serum VEGF concentrations show a positive correlation with the BMI and elevated levels occur in obesity that are reversed with weight loss (Miyazawa-Hoshimoto *et al.* 2003); also, the plasma VEGF is higher in genetically obese mice and those with adipocyte implants compared with nonobese mice (Miyazawa-Hoshimoto *et al.* 2005). In addition to these relationships with adiposity, VEGF can act synergistically with leptin in promoting the angiogenic process (Cao *et al.* 2001).

Insulin and angiogenesis

Pathological angiogenesis provides the vascular support that is essential for cancer progression. It is essential for the growth of a solid tumor beyond a few millimeters in diameter and for the development of distant metastases, and in consequence, a high level of angiogenic activity in breast cancer provides a biomarker of a poor prognosis (Uzzan *et al.* 2004). There is considerable interest in the potential for inhibitors of angiogenic factors as cancer therapeutic agents, but specifically targeting VEGF may be inadequate, even though it is a specific mitogen for vascular endothelial cells, given the redundancy in proteins with angiogenic properties (Ribatti 2011),

among which is insulin. Pathological angiogenesis is also responsible for some of the complications of type 2 diabetes, and its pharmacological inhibition is showing promise in the treatment of diabetic retinopathy and macular edema (Willard & Herman 2012).

Microvascular epithelial cells possess IRs, including those of vessels associated with breast cancer stromal tissues (Rensing *et al.* 2010), and insulin has its own direct stimulatory effects on vascular endothelial cell migration and capillary-like tube formation, which Liu *et al.* (2009) showed take place by activation of the PI3K/Akt pathway. Insulin can also enhance neovascularization indirectly: first, it can induce VEGF expression in vascular endothelial cells (Yamagishi *et al.* 1999, Jiang *et al.* 2003), which results in the autocrine stimulation of microvascular endothelial cell growth and tube formation and second insulin stimulates VEGF synthesis and its release from adipocytes (Mick *et al.* 2002, Fain & Madan 2005), which would provide for a paracrine interaction between the adipocytes of the peritumor adipose tissue and the tumor capsule and the tumor-associated vascular endothelial cells (Fig. 3). Both the PI3K/Akt and MAPK pathways are involved in this process, the two together being required for the sustained upregulation of VEGF under the influence of insulin (Jiang *et al.* 2003).

A similar endocrine action of insulin to enhance the paracrine promotion of angiogenesis by leptin is suggested by its known stimulation of leptin production in adipocytes combined with the proangiogenic activity of the adipokine itself (Fig. 3). The presence of a complex system of paracrine-mediated interactions involving insulin and the adipokines in the adipose tissue surrounding a breast cancer and the autocrine production of angiogenic factors by the tumor cells themselves suggests a particularly fruitful topic for future research. Of note is the report by Zhang *et al.* (2010) that angiogenesis became impaired around the periphery of solid tumors formed by breast cancer cells in the mammary fat pads of athymic nude mice when downregulation of the IR was induced while leaving IGF1 receptor expression intact and that this was associated with a diminished capacity for distant metastasis.

Estrogen production and bioactivity

Estrogens stimulate the proliferation of untransformed and neoplastic breast epithelial cells directly and also support tumor growth by promoting angiogenesis (Gupta & Kuperwasser 2006). Obese postmenopausal women have elevated levels of estrogens, which are

synthesized almost exclusively in adipose tissue stromal cells and can promote the development of ER-positive, estrogen-dependent, breast cancer by both endocrine and paracrine mechanisms (Vona-Davis & Rose 2007, Bulun *et al.* 2012); a similar situation holds in postmenopausal women with type 2 diabetes. PR expression by these ER-positive tumors is induced by estradiol, a response that was shown in MCF-7 cells to be dependent on insulin (Katzenellenbogen & Norman 1990). Panno *et al.* (1996) also showed that the arrest of MCF-7 cell growth in medium supplemented with serum stripped of steroids and protein growth factors was reversed by the addition of insulin or estradiol, that the two had synergistic stimulatory activity, and that insulin induced ER as well as PR expression and increased the binding capacity of the ER. Mauro *et al.* (2001) demonstrated that the expression and activation of ER enhanced insulin mitogenicity and that this was achieved by upregulation and enhanced function of IRS1 with increased PI3K/Akt and MAPK signaling.

Postmenopausal women with type 2 diabetes frequently have an increase in the plasma estrogens, which is distinct from any contribution arising from associated adiposity (Ding *et al.* 2007, Kalyani *et al.* 2009), and results from the enhancing effect of insulin on extraglandular estrogen biosynthesis. In the adipose tissue of women, but not men, insulin increases cytochrome P450 enzyme activity, so catalyzing the aromatization of the C19 steroid androstenedione to yield estrone, and also acts indirectly by enhancing the stimulatory effect of glucocorticoids on the aromatase enzyme system (McTernan *et al.* 2000); enzymatic reduction of this estrone then takes place to form estradiol.

In addition to the general stimulation of estrogen biosynthesis in the stromal cells of the visceral adipose tissue mass by insulin, which causes an elevation in the circulating estrogen levels and endocrine stimulation of ER-positive cells, there is the potential for paracrine stimulation originating specifically from high aromatase and 17- β -hydroxysteroid dehydrogenase activity in the stromal cells that form the tumor capsule. Furthermore, although not reported, it is feasible that insulin may stimulate the formation of estrogens from androstenedione in the breast cancer cells themselves, as these do express aromatase and 17- β -hydroxysteroid dehydrogenase (Sasano *et al.* 2006, Miki *et al.* 2007), so providing for autocrine stimulation of tumor cell proliferation.

Under physiological conditions, 30–50% of the plasma estradiol, the most biologically potent of the circulating estrogens, is bound to sex hormone-binding globulin (SHBG) and is functionally inert. Most of the

estradiol that is not associated with SHBG is weakly bound to albumin, from which it readily dissociates to become biologically available, as is the 1–2% that normally circulates free of any protein binding (Rose 1993). Low concentrations of SHBG occur in women with hyperinsulinemia and insulin resistance, when they indicate a high risk of type 2 diabetes (Lindstedt *et al.* 1991, Haffner *et al.* 1993). Elevated bioavailable estradiol and reduced SHBG levels in type 2 diabetes are independent of the BMI (Golden *et al.* 2007, Kalyani *et al.* 2009), as they are when present in association with increased postmenopausal breast cancer risk (Kaaks *et al.* 2005).

Commentary

The complex interacting mechanisms by which insulin may promote breast cancer development and progression are summarized in Fig. 4. Insulin is involved in the promotional stage of breast tumorigenesis and progression to expression of the metastatic phenotype, rather than initiation and neoplastic transformation of the breast epithelial cell. Type 2 diabetes-related breast cancer occurs typically in women beyond the menopause, but whether this is

due to a favorable hormonal environment, or the age at onset of, and length of exposure to insulin resistance, or that hyperinsulinemia is required for the promotion of initiated breast epithelial cells still remains uncertain. The question does have implications for breast cancer prevention because type 2 diabetes is occurring with increasing frequency in children and young adults. It does need to be stressed that the various events described in this review to make the connections between insulin action and the endocrine, paracrine, and autocrine activities of the adipokines have been established by laboratory experimentation, but their translational significance for human breast cancer requires further study.

Future studies should be directed at the interaction of environmental factors, such as the endocrine disturbances of obesity and type 2 diabetes, and genetic abnormalities. For example, variants of the gene that encodes transcription factor 7-like 2 (*TCF7L2*) have been associated with increased risk not only for type 2 diabetes (Zeggini & McCarthy 2007), but also for breast cancer (Burwinkel *et al.* 2006, Naidu *et al.* 2011). These relationships are supported by experimental studies showing that *TCF7L2* regulates hepatic glucose production (Norton *et al.* 2011), part of the

Figure 4 Insulin resistance-associated elevations in circulating insulin levels and insulin-inducible adipokine secretions in promoting breast cancer growth, invasion, and metastasis. These are achieved directly by the endocrine activity of insulin itself in stimulating insulin receptor-expressing tumor cell proliferation, survival, and metastatic capacity, enhancing estrogen action on tumor cells, and stimulating tumor-associated angiogenesis. Indirectly, insulin may increase these same effects on tumor behavior by stimulation of leptin and VEGF production, and suppression of adiponectin synthesis, in adipocytes with altered endocrine, paracrine, and autocrine actions by the adipokines. Full colour version of this figure available via <http://dx.doi.org/10.1530/ERC-12-0203>.

Wnt/ β -catenin signaling cascade, which contributes to pancreatic β -cell regulation (Le Bacquer *et al.* 2011).

Understanding the biochemical mechanisms by which insulin influences breast cancer progression offers some novel approaches to chemotherapy. Metformin, the standard first-line pharmaceutical treatment for type 2 diabetes, is currently undergoing evaluation as adjuvant therapy in breast cancer; in preclinical studies, it was shown to not only reduce elevated blood insulin concentrations but also to stimulate AMP-activated protein kinase activation in breast cancer cells, with inhibition of mTOR (Dowling *et al.* 2007), and to inhibit aromatase activity in breast adipose stromal cells (Brown *et al.* 2010). Downstream of the IR, several inhibitors of the PI3K/Akt pathway are under investigation in animal models and clinical trials (Gallagher *et al.* 2011, Hernandez-Aya & Gonzalez-Angulo 2011). How the potential therapeutic responses in breast cancer patients relate to the presence of insulin resistance and hyperinsulinemia is unclear, but in the nonobese MKR mouse with hyperinsulinemia and mild glucose intolerance, the mammary tumors show increased activation of the PI3K/Akt/mTOR pathway and pharmacological PI3K inhibition reduced tumor growth. Fierz *et al.* (2010) investigated the mTOR pathway in the MKR model and demonstrated that inhibition of mammary tumor growth occurs in mice treated with rapamycin.

We need a better understanding of the relationship between ER status, tumor estrogen dependence, and the coexistence of type 2 diabetes and its implications for breast cancer therapy. Although Michels *et al.* (2003) found type 2 diabetes to be a risk factor specifically for ER-positive postmenopausal breast cancer, and we have discussed the experimental support for an interaction between insulin and estrogens in the stimulation of breast cancer progression, Goodwin *et al.* (2012), when demonstrating an association between the fasting serum insulin, insulin resistance, and breast cancer prognosis, did not observe any modifying influence of ER status. The issue is one with important therapeutic implications. Gillespie *et al.* (2010) found that diabetes was associated with an increased risk for 'triple-negative' breast cancer, a tumor type that is negative for ER, PR, and HER2, possesses an aggressive metastatic phenotype, and has a poor prognosis. While this single report requires verification, the metabolic syndrome, with independent association for the blood glucose, has been associated with triple-negative breast cancer (Maiti *et al.* 2010).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

D P Rose participated in the drafting of this manuscript and he has seen and approved the final version. L Vona-Davis participated in the drafting of this manuscript and she has seen and approved the final version.

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