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Review

Molecular mechanisms of leptin and adiponectin in breast cancer

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ABSTRACT

Obesity is associated with an increased risk of breast cancer in postmenopausal women. Accumulating evidence suggests that adipose tissue, which is an endocrine organ producing a large range of factors, may interfere with breast cancer development. Leptin and adiponectin are two major adipocyte-secreted hormones. The pro-carcinogenic effect of leptin and conversely, the anti-carcinogenic effect of adiponectin result from two main mechanisms: a modulation in the signalling pathways involved in proliferation process and a subtle regulation of the apoptotic response. This review provides insight into recent findings on the molecular mechanisms of leptin and adiponectin in mammary tumours, and discusses the potential interplay between these two adipokines in breast cancer.

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1. Introduction

Obesity is related to many metabolic disorders like type 2 diabetes mellitus, coronary heart disease, and hypertension, and is associated with cancer development in different tissues including colon, prostate and breast.[1] It has been clearly demonstrated that obesity is a risk factor for breast cancer development in postmenopausal women.[2,3] Moreover, it is well established that an excess of adipose tissue favours metastasis development and breast cancer recurrence and is associated with a higher mortality.[4] Thus, overweight or obese women with breast carcinoma are 2.5 times as likely to die of their disease within 5 years of diagnosis compared with lean women.[5] Numerous factors have been suggested to clarify the relationship between obesity and breast cancer.

Obesity-associated hyperinsulinaemia and high circulating oestrogen levels may explain the interplay between adipocytes and mammary cancer cells.[6,7] In postmenopausal women, adipose tissue is the main source of aromatase (an enzyme that converts androgens to oestrogens) and an

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enhanced number and/or size of adipocytes in obese patients may amplify the androgen aromatisation. In addition, an excess of adipose tissue is associated with an increase in plasma levels of insulin and insulin-like growth factor-1 (IGF-1), which have mitogenic activities and are involved in breast tumour progression.[8] However, none of these hypotheses has been totally conclusive.

1.1. *Adipose tissue as an adipokine-secreting organ*

Accumulating evidence suggests that adipose tissue, which is an endocrine organ producing and secreting a large range of factors, may interfere with cancer development. These factors, called adipokines, include angiogenic factors, mitogens (leptin) and anti-mitogens (adiponectin), growth factors and pro-inflammatory cytokines (IL-1, TNF-alpha, IL-6) involved in the mediation and/or the coordination of inflammatory diseases and obesity.[9,10] Adipokines are produced by different fat depots, including sub-cutaneous, visceral and mammary adipose tissues. As expected, pathologies modifying adipose tissue biology, such as obesity, strongly alter adipokine production. As an example, obese subjects present decreased level of circulating adiponectin and increased plasma leptin concentration.[11,12] The alteration of adipokine secretion by adipose depots may explain the relationship between obesity and breast cancer development.

It is worth noting that adipokines may act on breast tissue in an endocrine manner (*via* external adipose depots), in a paracrine pathway (*via* mammary adipose tissue and non adipose sources including stromal cells and inflammatory cells) and in an autocrine action (*via* mammary tumour by itself). The structure of the mammary gland favours a close interaction between mammary adipose tissue and breast tissue, and suggests that adipokines produced by mammary adipose tissue and the tumour cell microenvironment may be the major link between obesity and disease progression and metastasis.[13–16]

1.2. *Adipokines and breast cancer*

Adipokines act *via* their receptors on mammary tumour cells to (a) influence tumour cell proliferation, migration and invasion in breast cancer; (b) regulate the production of epithelial-derived proteins, angiogenic proteins and growth factors; and (c) stimulate other cells in the tumour microenvironment to invade and proliferate. For example, a study reported a proliferative effect of adipocyte-secreted factors on MCF-7 breast cancer cell line, through the regulation of genes involved in cell motility, migration, survival, apoptosis and angiogenesis.[17] In the same way, Celis et al. identified in mammary adipose tissue 359 protein components and excreted factors that may give insight into the close interplay between mammary epithelium, stroma and fat tissue.[18] More recently, Perera et al. used a proteomic approach and described for the first time the secretion of epithelial-derived proteins in MCF-7 cells in response to leptin.[19] This adipokine and adiponectin are the two major adipocyte-secreted hormones. We have recently explored simultaneously the expression of leptin and adiponectin both in breast cancer cell lines and in mammary tissue of ductal carcinoma and suggested antagonistic properties of these two adipokines in breast cancer development.[20,21] Here we review in details the *in vitro* and *in vivo* molecular mechanisms by which leptin induces and, conversely, adiponectin represses tumour proliferation in breast cancer cells.

2. Leptin: a pro-carcinogenic adipokine?

2.1. *In vitro data*

Leptin receptor (Ob-R) mRNA and protein expression have been characterised in different breast cancer cell lines, including MCF-7, MDA-MB-231 and T47D.[21–25] The effect of leptin, acting *via* its receptor, on cell growth is well known, with leptin stimulating proliferation of MCF-7, SK-BR-3, T47D and ZR-75-1 breast cancer cells (Table 1).[21,22,25–27]

In vitro experiments show that the proliferative activity of leptin is mediated *via* different signalling pathways (Fig. 1). First, leptin induces the PI3K/Akt survival pathway by activating the phosphorylation of Akt Thr308[28] or Akt Ser473[23,29] and by stimulating the protein expression of PKC-alpha, which is controlled by PI3K.[30] Second, leptin activates the MAPK pathway by inducing ERK1 and ERK2 phosphorylation.[22,29,31] Third, leptin rapidly and directly stimulates the STAT3 pathway and the up-regulation of c-myc (one target gene of STAT3), at both mRNA and protein levels.[27,31,32] Leptin also up-regulates cdk2 and cyclin D1 (genes promoting cell cycle transition G1 → S), indicating that cell proliferation may be activated through the alteration of cell checkpoints that accelerate cell cycle progression.[30] Similar results were obtained in ZR-75-1 breast cancer cells, where leptin-induced proliferation is also associated with enhanced expression of c-myc and cyclin D1.[33] In 4T1 mouse mammary cancer cells, leptin treatment increases not only the expression of cyclin D1, but also that of VEGF and its receptor VEGF-R2, suggesting leptin may promote angiogenesis in breast cancer through VEGF signalling.[34] Recently, Perera et al. have identified more than 64 leptin-regulated genes, including those for growth factors, cell cycle regulators, extracellular matrix (ECM) proteins and genes associated with metastasis.[35]

In breast cancer, the pro-carcinogenic effect of leptin results not only from an enhanced activity of signalling pathways involved in proliferation process but also from a probable down-regulation of the apoptotic response (Table 1). When MCF-7 cells are incubated for a long time (>24 h) with leptin, a significant reduction in p53 expression is observed at both mRNA[21] and protein levels.[28] A reduction in Bax production is also observed.[28] Accordingly, when the effect of leptin on breast cancer cells was assessed by TUNEL assay, leptin-treated MCF-7 cells demonstrated a 6-fold reduction in apoptosis compared with controls.[35] In parallel, leptin up-regulates the expression of anti-apoptotic genes Bcl2 and survivin.[35] It is worth noting that leptin also suppresses docetaxel-induced apoptosis by inhibiting caspase-9 activity, raising the possibility for leptin to counteract the beneficial action of chemotherapeutic drugs.[36] Similarly, in the Bcl2-negative cell line ZR-75-1, leptin-induced proliferation is associated with inhibition of p53 and $p21^{WAF1/C1P1}$ expression.[33] In MDA-MB-231 ERα negative human breast cancer cell line, leptin stimulates cell growth and this effect was mediated *via* STAT3 and/or ERK1/2 signalling pathways.[37] But surprisingly,

Table 1 – Effects of leptin on breast cancer cells *in vitro*.

Cell line	Incubation time	Concentration (ng/ml)	Proliferative effect	Ref.
MCF-7	24 h	800	Yes	[22]
	5 d	1600	Yes	[30]
	24 h	4	No	[26]
	24/72 h	100	Yes	[29]
	24/48/72 h	1600	Yes	[27]
	24 h	100	No (anti-proliferative)	[42]
	48–96 h	1000	Yes	[21]
T47D	48 h	100	Yes	[40]
	24 h	50–100	Yes	[41]
	24/48/72 h	320	Yes	[27]
	24/48 h	50–100	Yes	[42]
ZR-75-1	24 h	4	Yes	[26]
	48 h	25	Yes	[33]
MDA-MB-231	24/48 h	50–100	Yes	[42]
	48 h	320–1600	Yes	[37]
MDA-MB-361	24/48 h	10–100	Yes	[42]
SKBR3	24/48 h	5–50	Yes	[42]
Cell line	**Incubation time**	**Concentration (ng/ml)**	**Cell cycle progression**	**Ref.**
MCF-7	72 h	16,000	Yes	[30]
	24 h	500	Yes	[35]
	48 h	50	No	[28]
T47D	48 h	50	No	[28]
ZR-75-1	48 h	100	Yes	[33]
MDA-MB-231	24/48 h	640	No	[37]
Cell line	**Incubation time (h)**	**Concentration (ng/ml)**	**Anti-apoptotic effect**	**Ref.**
MCF-7	24 h	500	Yes	[35]
	24 h	ND	No	[30]
	48 h	50	No	[28]
T47D	48 h	50	No	[28]
ZR-75-1	48 h	100	Yes	[33]
MDA-MB-231	24/48 h	640	No	[37]

when leptin is combined with cAMP elevating agents (that inhibit breast cancer cell proliferation), there is a strong increase of Bad/Bcl2 protein ratio (mainly due to a dramatic down-regulation of Bcl2 content), followed by caspase-3 activation and PARP cleavage, leading to apoptosis induction.[37] Additionally, this leptin-induced apoptosis is accompanied by the decrease of cyclin D1 and A and by the increase of inhibitor $p27^{kip1}$ cell cycle regulating protein levels, leading to cell cycle arrest at G1 phase. Thus, in some cases, leptin can potentiate the action of anti-proliferative compounds and may exert opposite actions related to apoptosis process, depending on the model studied.

The leptin-mediated proliferation involves a possible interaction between leptin and oestrogen systems to promote breast carcinogenesis.[38] For example, leptin exhibits oestrogen-producing activity by enhancing aromatase mRNA expression, aromatase protein content and its enzymatic activity and enhances the sensitivity of breast cells to oestrogens *via* up-regulation of ERα in MCF-7 breast cancer cells.[31,39] In addition, the effect of both leptin and oestradiol on ZR-75 cell proliferation is higher that leptin or oestradiol alone.[33] Recently, Binai et al. demonstrated that ERα expression enhanced leptin-induced STAT3 transactivation activity and leptin-induced cell viability in MDA-MB-231 cells transfected with HEGO cDNA construct to overexpress ERα.[32] Interestingly, leptin interfered with the effects of the anti-oestrogen ICI 182,780 in MCF-7 cells by increasing nuclear ERα expression and proliferation, decreasing ERα degradation and inducing ERα dependant transcription from oestrogen response element-containing promoters.[29] However, numerous *in vitro* studies carried out with breast cancer cell lines have found a proliferative effect of leptin in ER-positive cell lines[21,22,26,40,41] as well as in ER-negative cell lines.[42] These data suggest that leptin-induced cell proliferation cannot be explained only by oestrogen-dependent mechanisms.

2.2. *Animal studies*

Animal studies support a role for leptin in mammary tumour development (Table 2). Mice deficient in leptin, Lep –/– (ob/ob), did not develop transgene-induced mammary tumours whereas non obese mice Lep +/+ and Lep +/– developed

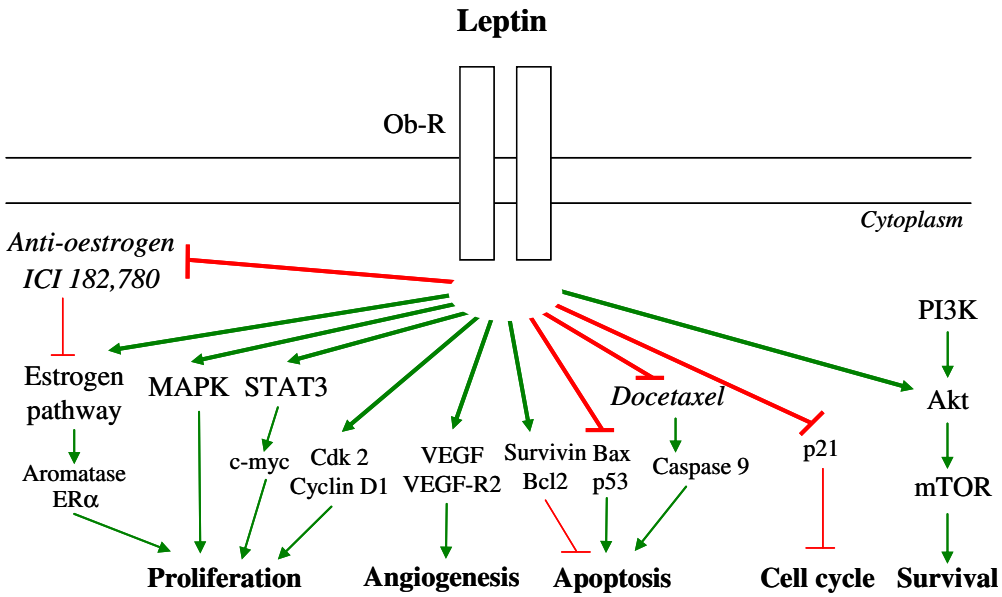

Fig. 1 – Signalling pathways of leptin in breast cancer cell. Ob-R: leptin receptor; STAT3: signal transducer and activator of transcription 3; Cdk2: cyclin-dependant kinase 2; ERα: oestrogen receptor α; MAPK: mitogen-activated protein kinase; Bcl2: B-cell lymphoma 2; Bax: Bcl2 associated X protein; PI3K: phosphoinositide 3-kinase; mTOR: mammalian target of rapamycin; and VEGF: vascular endothelial growth factor.

tumours in 50% and 67% of cases, respectively.[43] In a similar experiment, the same authors showed that mice deficient in leptin receptors, Lepr –/– (db/db), did not develop transgene-induced mammary tumours whereas non obese mice Lepr +/+ and Lepr +/– developed tumours in 69% and 82% of cases, respectively.[44]

Other authors confirmed the *in vivo* proliferative effect of leptin exposure using MCF-7 xenografts in nude mice and showed that leptin increases tumour volume by 100% after 13 weeks of treatment.[45] Tumour analysis revealed a hyper-activation of MAPK and STAT signalling pathways.

In order to assess the therapeutic potential of the leptin pathway, the activity of a leptin receptor antagonist peptide (LPrA2) was evaluated in mice. A sub-cutaneous injection of LPrA2 prior to inoculation with mouse (4T1) or human (ER-positive MCF-7 and ER-negative MDA-MB-231) mammary cancer cells delayed the development and slowed the growth of mammary tumours.[34,46] This effect was observed along with a reduced expression of pro-angiogenic (VEGF/VEGF-R2, leptin/Ob-R, and interleukin 1 receptor type I, IL-1RtI) and pro-proliferative molecules (proliferating cell nuclear antigen, PCNA and cyclin D1).[46]

Table 2 – Effect of leptin (Lep) on breast cancer cells *in vivo*.

Mouse model	Effect (*versus* respective controls)/molecular mechanisms	Ref.
• TGF-α/Lep –/– (ob/ob) mice	No transgene-induced mammary tumour development	43
• TGF-α/Lep +/+ non obese mice	Tumour development in 50% of cases	43
• TGF-α/Lep +/– non obese mice	Tumour development in 67% of cases	43
• TGF-α/Lepr –/– (db/db) mice	No transgene-induced mammary tumour development	44
• TGF-α/Lepr +/+ non obese mice	Tumour development in 69% of cases	44
• TGF-α/Lepr +/+ non obese mice	Tumour development in 82% of cases	44
• Nude mice with MCF-7 xenograft leptin-treated	Increase in tumour size by 100% after 13 weeks of treatment → hyper-activation of MAPK and STAT signalling pathways	45
• Nude mice with MCF-7 xenograft LPrA2-treated	Decrease in tumour growth by 90% after 3 weeks of treatment	46
• Nude mice with MDA-MB231 xenograft LPrA2-treated	Decrease in tumour growth by 50% after 3 weeks of treatment → reduced expression of pro-angiogenic and proliferative molecules	46
• 4T1 tumour-bearing mice LPrA2-treated	Decrease in tumour growth by 90% after 3 weeks of treatment → reduced expression of VEGF and VEGF-R2	34
• MMTV-neu (strain 202) mice with BC xenografts and with elevated leptin (fed with high-fat diet)	No impact on tumour growth or metastasis	47

Table 3 – Effects of adiponectin on breast cancer cells *in vitro*.

Cell model	Incubation time	Concentration (ng/ml)	Anti-proliferative effect	Ref.
MCF-7	4–6 d	10,000	No	[64]
	48 h	50–5000	Yes	[62]
	24/48/72/96 h	10,000	Yes	[21]
	24 h	2.5–250	Yes	[58]
T47D	48 h	20,000	Yes	[63]
	48 h	200–5000	Yes	[62]
	24/48/72 h	10,000	Yes	[55]
SKBR3	48 h	500–5000	Yes	[62]
MDA-MB-231	48 h	50–5000	No	[62]
	24/48/72 h	10,000	Yes	[55]
	24 h	25	Yes	[56]
MDA-MB-361	48 h	50–5000	No	[62]
MCF-10a	4–6 d	10,000	Yes	[64]
Cell model	**Incubation time**	**Concentration (ng/ml)**	**Cell cycle inhibition**	**Ref.**
MDA-MB-231	48 h	15,000	Yes	[57]
	48 h	10,000	Yes	[55]
T47D	48 h	10,000	Yes	[55]
Cell model	**Incubation time**	**Concentration (ng/ml)**	**Apoptotic effect**	**Ref.**
MCF-7	96 h	250	Yes	[58]
	48 h	ND	No	[59]
	30 min	5000	Yes	[28]
T47D	48 h	15,000	No	[57]
	48 h	20,000	No	[63]
	48 h	10,000	No	[55]
MDA-MB-231	96 h	ND	Yes	[60]
	48 h	15,000	Yes	[57]
	24 h	25–250	No	[56]
	48 h	10,000	No	[55]

The impact of diet-induced obesity was determined in MMTV-neu mice which develop ER-negative mammary tumours.[47] Although a high-fat diet resulted in elevation of serum leptin levels, the different diet-dependant alterations in leptin level had no effect on tumour incidence and metastasis. This animal model suggests that obesity is more likely to be associated with the development of ER+ and/or hormone-responsive breast cancers, consistent with observations in postmenopausal women.

2.3. *Human studies*

In a previous study, we have investigated the expression of leptin *in vivo* in breast tumour tissues and identified leptin as a proliferative factor in human breast carcinoma.[48] Moreover, we reported that leptin is not expressed in healthy breast tissue but is detected in normal tissue adjacent to ductal carcinoma, suggesting leptin may be a diagnostic marker of early tumorigenicity in human ductal breast carcinoma.[48] More recently, we have refined the localisation of leptin and Ob-R in mammary tissue and confirmed its implication in tumour development.[38] Our study, and others, observed a co-expression of leptin and Ob-R in primary breast cancer, showing that leptin acts on mammary tumour cells *via* an autocrine pathway.[38,49,50] Recently, a study conducted on 517 breast cancer patients showed a positive correlation between leptin expression and Ki-67 labelling index, in accordance with the *in vitro* proliferative activity of leptin.[51] However, such a correlation was not observed in two other studies based on 35 and 148 breast cancer patients, respectively.[38,49]

Leptin may also promote mammary tumour growth through multiple mechanisms such as a modulation of the extracellular environment, a down-regulation of apoptosis and/or an up-regulation of anti-apoptotic genes. Indeed, immunohistochemical analysis of breast cancer biopsies revealed statistically significant positive correlation between leptin and anti-apoptotic protein Bcl-xL (but not Bcl2 protein) as well as between leptin and pro-apoptotic Bak and Bax.[52] The same authors also found a significant positive correlation between Ob-R and Bcl-xL and Bax. Thus, leptin is clearly associated with signalling pathways related to apoptosis. However, *in vivo* observations do not always reflect the *in vitro* results and functional relationships between leptin system and apoptotic proteins are not clearly understood yet.

We also found that Ob-R expression in primary breast carcinoma was positively correlated with ERα expression,[38] suggesting a possible interaction between leptin and oestrogen systems to promote breast carcinogenesis, as demonstrated

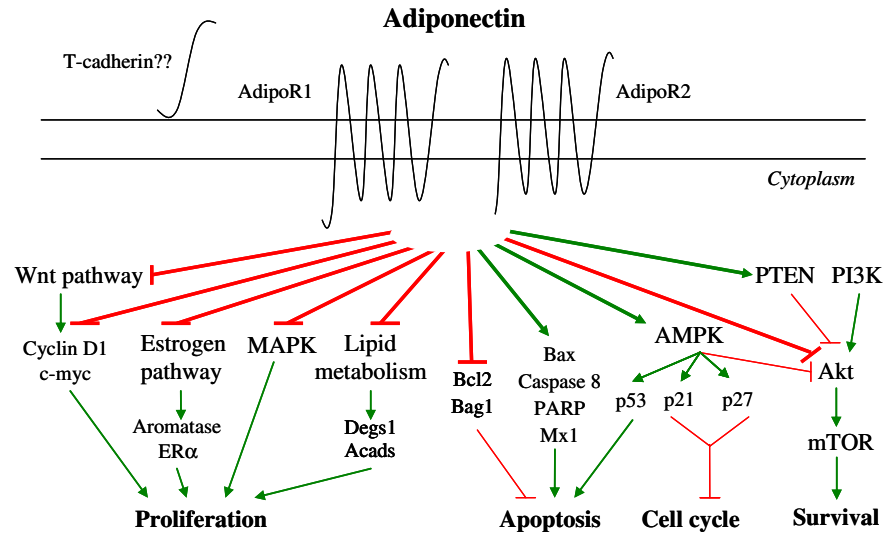

Fig. 2 – Signalling pathways of adiponectin in breast cancer cell. AdipoR1: adiponectin receptor 1; AdipoR2: adiponectin receptor 2; ERα: oestrogen receptor α; MAPK: mitogen-activated protein kinase; DEGS1: degenerative spermatocyte homologue 1, lipid desaturase; ACADS: acyl-coenzyme A dehydrogenase, C-2 to C-3 short chain; Bcl2: B-cell lymphoma 2; Bag1: Bcl2-associated athanogene 1; Bax: Bcl2 associated X protein; PARP: poly (ADP-ribose) polymerase; Mx1: myxovirus resistance protein 1; AMPK: AMP-activated protein kinase; PI3K: phosphoinositide 3-kinase; and mTOR: mammalian target of rapamycin.

in different *in vitro* studies. This result is consistent with that of Revillion et al., who noted a positive relationship with Ob-R mRNA and ER expression in primary breast cancer.[53] Garofalo et al. observed a similar relationship in lymph node metastases, but not in primary tumours.[49]

3. Adiponectin: an anti-carcinogenic adipokine?

3.1. *In vitro data*

Adiponectin receptors (AdipoR1 and AdipoR2) mRNA and protein expression have been characterised in different breast cancer cell lines, including MCF-7, MDA-MB-231, SKBR3 and T47D.[21,54,55] However, the effective receptor of adiponectin activity in breast cancer cells is not clearly defined. Nakayama et al. noted that siRNA against AdipoR1 completely abrogated the growth inhibition of adiponectin in T47D breast cancer cells and limited to about 50% the anti-proliferative activity of adiponectin in MDA-MB-231 breast cancer cells.[55] In contrast, two studies reported that siRNA against AdipoR1, AdipoR2 or both had no or little effect on adiponectin-mediated inhibition of the proliferation in MDA-MB-231 cells.[56,57] These results suggest that the activity of adiponectin in breast cancer cell line may be mediated partly by AdipoR1 and AdipoR2, but also by others receptors (such as T-cadherin), or *via* alternative molecular/signalling pathways.

Numerous studies have assessed the activity of adiponectin on cell growth and documented the anti-proliferative potential of adiponectin in various breast cancer cell lines,[57] including MCF-7, MDA-MB-231 and T47D (Table 3).[55,58–60] Recently, we showed that 24-h adiponectin treatment reduced MCF-7 cell proliferation, and this inhibition was observed up to 96 h.[21] A summary of the various signalling pathways by which adiponectin accomplishes its different anti-proliferative activities is shown in Fig. 2.

Adiponectin-repressed proliferation in breast cancer cells is mediated through an inactivation of p44/42 MAPK protein 1 and 3 expression, a stimulation of AMPK activity by phosphorylation at Thr172 and a decrease in Akt phosphorylation (Thr308) associated with an increased expression of LKB1 leading to a reduction of mTOR activity as evidenced by reduced phosphorylation of S6K.[28,58,61] In MDA-MB-231 cells, a prolonged treatment with adiponectin can modulate the glycogen synthase kinase-3β (GSK-3β)/β-catenin signalling pathway by blocking serum-induced phosphorylation of Akt and GSK-3β and suppressing intracellular accumulation of β-catenin and its nuclear activities, which consequently reduces cyclin D1 expression.[55,57] In MCF-7 cells, Dieudonne et al. also reported a down-regulation in cyclin D1 and c-myc protein expression.[58] In contrast, other authors found that cyclin D1 protein expression is increased in MCF-7 and T47D in response to adiponectin for 24 h,[62] suggesting a biphasic effect of adiponectin on cyclin D1 expression. Recently, we have shown by qRT-PCR and microarray analysis that in MCF-7 cells, adiponectin represses a large number of genes involved in proliferation, among which are those involved in cell cycle activation (mitogen-activated protein kinase 3, MAPK3) and fatty acid metabolism (acyl-coenzyme A dehydrogenase, ACADS and degenerative spermatocyte homologue 1 lipid desaturase, DEGS1).[21]

Depending on the cell lines studied and the incubation time, adiponectin induces cell apoptosis[28,58,60] or not,[55,56,59,63] but it is worth noting that apoptosis was more likely observed when incubation time was longer than 48 h (Table 3). In addition, increased PARP cleavage was detected

in all of the ER-positive cell lines tested but not in the ER-negative cell lines.[62] Importantly, Dos Santos et al. found that a 24 h adiponectin incubation has no effect on apoptosis while modulating expression of apoptotic biomarkers in MDA-MB-231 cells.[56] In this study, Bcl2 was down-regulated whereas p53 and Bax were up-regulated. Thus, modulation of apoptotic biomarkers which is an early event occurring before the apoptosis process can be detected. Very little *in vitro* regulation was observed for Bcl2 family members although Bax protein expression was increased in MCF-7 and MDA-MB-231 cells in response to adiponectin.[56,58] The levels of cleaved caspase-3, caspase-6, and caspase-9 were not altered in MCF-7 cells but adiponectin activated caspase-8, raising the possibility for this adipokine to mediate apoptosis through the extrinsic pathway.[62] In another study, microarray data revealed a 5-fold increase of transcript levels of caspase-1 in normal breast epithelial cell line MCF-10A after treatment with adiponectin whereas such a treatment does not affect caspase-1 levels in MCF-7 cells.[64] Finally, we have shown that, in MCF-7 cells, adiponectin represses the expression of Bcl2 associated athanogene (Bag1), involved in apoptosis inhibition.[21]

Some data suggest a possible interaction between adiponectin and oestrogen pathways. For example, adiponectin is able (i) to reduce aromatase and oestrogen receptor (ER) mRNA expression in breast cancer cell[21]; (ii) to impede significantly oestradiol-induced cell proliferation[58]; and (iii) to stimulate mRNA expression of oestrogens β2 and β5, known to form heterodimers with α-receptor, which consequently inhibits the transcriptional activity of this receptor.[64] However, the *in vitro* anti-proliferative effect of adiponectin has been extensively reported on ER-positive[21,58,59,63] as well as on ER-negative breast cancer[55,56,60,62] cell lines, suggesting that adiponectin-repressed tumour proliferation cannot be explained only by oestrogen-dependent mechanisms.

3.2. *Animal studies*

To date, only few *in vivo* studies assessed the role of adiponectin in breast carcinogenesis in animal models (Table 4). In one report, the authors showed that both pre-treatment of cells with recombinant adiponectin and adenovirus-mediated over-expression of this adipokine substantially reduced the mammary tumorigenesis of MDA-MB-231 cells in female nude mice in terms of tumour size and tumour weight, 35 d after tumour injection.[57] The action of adiponectin could be mediated *via* a cross-talk between the PI3K/Akt and the canonical Wnt/β-catenin signalling pathways. Indeed, adenovirus-mediated over-expression of adiponectin substantially enhanced WIF1 (Wnt inhibitory factor-1) expressions in MDA-MB-231 tumours implanted in nude mice and inhibition of Wnt signalling by over-expression of WIF1 is known to inhibit Akt activity.[65]

Moreover, when mice receive an intravenous injection of recombinant adenovirus expressing adiponectin, the observed increase in circulating adiponectin level is followed by a decrease in tumour size, as soon as 2 weeks after injection, and by a decrease in tumour weight (–42.5%) at the end of the experiment.[57] Thus, adiponectin may have a systemic effect as well.

In a more recent paper, using a model of transgenic mice with normal PyVT(+/–)/ADN(+/+) or reduced PyVT(+/–)/ADN(+/–) adiponectin expressions, the authors found an *in vivo*-reduced production of adiponectin associated with earlier tumour onset and accelerated tumour growth.[66] This tumorigenesis in mammary cancer cells occurred by down-regulation of tumour suppressor PTEN activity and hyper-activation of PI3K/Akt signalling pathway. Furthermore, phosphorylations of both Akt at serine 473 and GSK-3β at serine 9 were significantly increased in primary tumour cells derived from PyVT(+/–)/ADN(+/–) mice. On the other hand, the phosphorylation of ERK1/2 was not different between the two types of tumour cells from PyVT(+/–)/ADN(+/+) and PyVT(+/–)/ADN(+/–) mice. The protein levels of β-catenin and its target cyclin D1 were largely elevated. The induction of β-catenin signalling was also confirmed by measuring its nuclear activities, which were increased by 4.5-fold in PyVT(+/–)/ADN(+/–) tumour cells.

Since adiponectin is an inhibitor of angiogenesis *in vivo*,[67] one may suggest that low adiponectin levels in breast cancer might contribute to promote the formation of new vessels necessary for tumour growth. However, adiponectin may have a possibly biphasic effect on tumour progression since *in vivo* data showed that mice lacking adiponectin have a delayed angiogenic response, suggesting adiponectin has potent angio-mimetic properties in the early steps of tumour vascularisation.[68] The authors hypothesised that increased risk and poorer prognosis seen in breast cancer patients with low serum adiponectin levels (such as obese women) could be due to acquired adaptation of tumours to anti-angiogenic stress. Thus, this stress may trigger an adaptive reaction that fuels tumour growth at later stages. The pro-angiogenic effect of

Table 4 – Effect of adiponectin (Adi) on breast cancer cells *in vivo*.

Mouse model	Effect (*versus* respective controls)/molecular mechanisms	Ref.
• Nude mice with MDA-MB-231 xenograft pre-treated with recombinant Adi	Decrease in tumour weight by 58% after 35 d → PI3K/Akt/GSK-3β and canonical Wnt/β-catenin pathways	57
• Nude mice with MDA-MB-231 xenograft injected with adenovirus over-expressing Adi	Decrease in tumour weight by 32% after 35 d	57
• MMTV-PyVT mice/Adi haplodeficient	Earlier tumour onset and accelerated tumour growth → PI3K/Akt/β-catenin hyper-activation, PTEN inactivation	66
• MMTV-PyVT/Adi null mice	Delayed angiogenic response	68
• MMTV-PyVT/Adi null mice	Increase in hypoxia and apoptosis	69

adiponectin was unexpectedly concomitant with increased hypoxia and apoptosis in adiponectin knock-out mammary tumours.[69]

3.3. *Human studies*

We recently investigated by immunohistochemistry the adiponectin expression in biopsies of breast cancer epithelial tissues.[20] We found that only 15% of the tumour tissues are positive for adiponectin, suggesting a weak autocrine/paracrine local implication for this adipokine in breast cancer cells. Similarly, adiponectin mRNA was not or weakly expressed in breast cancer tissues.[54,63] In contrast with *in vitro* results, we noted by immunohistochemistry that adiponectin receptors were co-expressed in only 15% (7 out of 45) of invasive ductal carcinoma and that AdipoR2 was the predominant receptor of adiponectin in breast cancer tissues (observed in 82% of invasive breast cancer).[21] Korner et al. noted that AdipoR1 and AdipoR2 were expressed in about 25–30% of breast cancer tissues.[63] Interestingly, AdipoR1, but not AdipoR2, is expressed in stromal cells, suggesting that adiponectin may affect stromal cells through AdipoR1.[54] Recently, Pfeiler et al. observed an inverse correlation between AdipoR1 expression and size in *in situ* ductal carcinoma tissues, suggesting that loss of AdipoR1 favours progression of the preinvasive lesion.[70] Finally, we noted that AdipoR2 expression was more pronounced in malignant cells than in normal breast tissue.[21] These data support the hypothesis of functional differences between AdipoR1 and AdipoR2 in breast cancer, and thereby, distinct signalling pathways may be activated by each adiponectin receptor *in vivo*.

4. Interplay between leptin and adiponectin

As demonstrated above, many studies have addressed the role of leptin or adiponectin in breast cancer development, but little attention has been paid to the potential interplay between them.

We recently documented the expression of adiponectin, leptin and their respective receptors in MCF-7 cells and found the mRNA level of leptin was markedly higher than adiponectin mRNA level,[21] suggesting that leptin should be more abundant in the tumour and in the tumour microenvironment. These results were confirmed on human breast cancer biopsies by immunohistochemistry, since leptin expression was noted in 80% of invasive ductal carcinoma whereas adiponectin was only detected in 15% of breast cancer.[20]

In addition, these two adipokines may down-regulate each other's pathway, adiponectin treatment decreasing leptin and Ob-R mRNA expression and leptin inhibiting AdipoR1 mRNA expression in MCF-7 cells.[21] Similarly, Dos Santos et al. observed the down-regulation of both adiponectin receptors induced by leptin in MDA-MB-231 cancer cells.[56] These results suggest that obesity- associated hypoadiponectinemia and hyperleptinemia should increase the leptin sensitivity of breast cancer cells.

The effect of both adiponectin and leptin on breast cancer cell proliferation is not well understood. We observed that MCF-7 cell proliferation following a 96 h treatment with leptin can be significantly reversed by adiponectin, using the same concentration for both adipokines (1 μg/ml).[21] Recently, Nkhata et al. noted that the effect of the ratio between adiponectin (5 μg/ml) and leptin (50 ng/ml) treatment was cell line dependant, inducing an inhibitory response on MCF-7 and T47D ERα positive cells, a proliferative response on MDA-MB-361 ERα positive cells and no effect on MDA-MB-231 and SK-BR-3 ERα negative cells.[28] At these concentrations, breast cancer cell lines expressing the ERα were adipokine responsive cells, suggesting that the effects observed could be mediated in part by the oestrogen pathway and that, adiponectin or leptin could impose their anti- or pro-proliferative activity depending on the cell line. In the same way, Grossmann et al. analysed the relationship between adipokines and oestrogen pathway using MDA-MB-231 cells transfected (MDA-ERα) or not (MDA-wt) with ERα.[71] Globular adiponectin (2.5 μg/ml) and leptin (32.8 μg/ml) co-incubation reduced the cell proliferation and the MDA-ERα cells were more sensitive as compared to ER-negative MDA-wt cells.

The anti-proliferative activity of adiponectin/leptin treatment on MCF-7 and T47D cells was mediated *via* the down-regulation of the activated Akt (Thr308) protein level, pathway known to be involved in the cell survival.[28] The activation of p-Akt protein levels following a 48 h treatment with leptin was significantly reversed by adiponectin below basal levels in MCF-7 cells. Nkhata et al. also found that activated MAP kinase (p-p42/44 MAPK) was barely detectable with leptin treatment (0.3-fold of basal), although the levels of this protein appeared to be restored to basal levels when cells were co-treated with leptin and adiponectin for 48 h in MCF-7 cells.[28]

The effect of the adiponectin/leptin treatment on apoptosis is unclear. Nkhata et al. observed that the incubation of both adiponectin (5 μg/ml) and leptin (50 ng/ml) for 48 h increased the number of apoptotic MCF-7 cells while the number of apoptotic T47D cells is decreased.[28] In MCF-7 cells, a 30 min adiponectin/leptin co-treatment up-regulated p53 and Bax expressions but these two proteins were down-regulated after a 48 h co-treatment. In T47D cells, p53 and Bax protein expressions were inhibited at both time points (30 min and 48 h). These results suggest a very subtle regulation in the molecular mechanisms by which the different adipokines regulate breast tumour cell proliferation and apoptosis.

Taken altogether, these data show that leptin and adiponectin may cross-talk by influencing each other's regulation on mitogenic and/or apoptotic signalling pathways.

5. Conclusion

Leptin and adiponectin are adipokines secreted by adipose tissue and also by epithelial tissue of breast tumour. The antagonism between adiponectin and leptin has been well documented in metabolic diseases and obesity but not in human breast cancer. The data summarised in this review demonstrate a major role for leptin and adiponectin in breast cancer progression based on their molecular mechanisms. In particular, leptin enhances breast cancer cell proliferation by inhibiting pro-apoptosis signalling pathways and by favouring *in vitro* sensitivity to oestrogens. Despite the increasing

evidences of association between metabolic syndrome and triple-negative (ER-negative/PR-negative/HER2-negative) breast cancers[72], the role of leptin on ER-negative breast cancers is still poorly understood and remains to be elucidated. In addition to its *in vitro* proliferative activity, leptin stimulates tumour growth in animal models. Conversely, adiponectin displays *in vitro* anti-proliferative, pro-apoptotic and anti-oestrogen properties. Moreover, adiponectin is involved in tumour size reduction in animals and low plasma levels of adiponectin are associated with higher risk of breast cancer progression in women. Importantly, if adipokines clearly modulate the apoptotic response *in vitro*, they may exert their activities through different pathways from a cell type to another since breast cancer cell lines display distinct patterns of apoptosis-regulatory genes.[73] For example, MCF-7 cells are caspase-3 negative, ZR-75 cells are Bcl2 negative and the p53 status is either wild or mutant, depending on the cell line.

Metabolic dysregulations associated with obesity (such as hypoadiponectomia and hyperleptinemia) are likely to promote cancer cell growth *via* both systemic and local mechanisms. When mammary cells are engaged in the process of carcinogenesis, they produce adipokines (mainly leptin) able to act on surrounding cancer cells in a paracrine and/or autocrine manner. Although little attention has been paid to the potential interplay between leptin and adiponectin, emerging data suggest the ratio of these adipokines may be more important in breast cancer than their absolute concentrations.[74] Consequently, there is increasing evidence that targeting the adiponectin:leptin ratio might be a new prognostic and/or therapeutic strategy for postmenopausal breast cancer. Hence, the understanding of molecular mechanisms of both leptin and adiponectin and their interplay in breast cancer offer the prospect of new therapeutic approaches targeting similar signalling pathways.

Conflict of interest statement

None declared.

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