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Research Article

The Effects of the Obesity-Related Hormones Leptin, Insulin, IGF-I and Adiponectin on Ishikawa and HEC-1A, MCF-7 and SKOV-3 Cancer Cell Lines

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Abstract

Background: Understanding how obesity influences the activities of dysregulated hormones in overweight women is crucial for managing the increased risk of cancer associated with obesity. Thus, this study aimed to investigate the response of cancer cell lines to obesity- related hormones *in vitro*.

Materials and Methods: The study focused on two endometrial cell lines (Ishikawa and HEC-1A), one breast cell line (MCF-7), and one ovarian cell line (SKOV-3). The effects of four obesity-related peptides known to have tumorigenic properties, namely leptin, insulin, IGF-I, were examined. Additionally, the study explored the impact of adiponectin, an anti-tumorigenic hormone.

Results: The study revealed the presence of hormone receptors in all four cell lines. The tumorigenic hormones demonstrated cell line-specific increases in cell proliferation, although the response was minimal in the SKOV-3 ovarian cell line compared to the endometrial and breast cancer cell lines. Notably, adiponectin exhibited a dual effect on cancer cell growth. At low concentrations, it promoted growth, while at high concentrations, it had the opposite effect. The study also observed that alterations in apoptotic levels in cancer cells induced by hormones did not consistently correlate with their effects on cell number or VEGF secretion. Interestingly, in most cases, VEGF secretion was regulated in the opposite direction to proliferation by the peptides.

Conclusion: Overall, this study underscores the tissue-specific oncogenic effects of the hormones examined, which could be influenced by genetic variations among the four cell lines. It is important to note that the study was conducted *in vitro*, but the findings offer valuable insights into the potential involvement of obesity-related hormones in cancer advancement. The research implies that additional investigations will contribute to a better comprehension of the intricate interactions uncovered. Elucidating the network connecting hormones, cancer cells, and the tumour microenvironment *in vivo* will have significant clinical implications.

Keywords: *Adiponectin; Endometrial Cancer; Insulin; Insulin-Like Growth Factor-1; Leptin; VEGF*

Introduction

A wealth of evidence states that obesity is a risk factor for increased cancer incidence. Obese individuals may produce altered levels of cancer-promoting hormones compared to non- obese women, including leptin, insulin, insulin-like growth factor-I (IGF-I) and adiponectin. However, the potential roles of these hormones in cancer growth have not been well addressed. *In vivo*, white adipose tissue is the primary

source of the leptin hormone and the major determinant of the level of the circulating hormone. However, many other organs also produce leptin [1]. It has been observed that leptin concentrations in obese women are higher than in non-women [2,3], and elevated leptin concentrations have been positively associated with endometrial cancer [4]. Further, *in vitro* studies show increased cell proliferation [4,5] of endometrial cancer cells after stimulating with leptin.

Insulin is produced in the islets of Langerhans in the pancreas and plays a crucial role in glucose regulation. IGF-I is a hormone with a structure closely related to that of insulin and is synthesised mainly in the liver. Increased body fat is associated with higher insulin levels, possibly due to adipocyte-derived signals [6]. It has been observed that serum insulin levels are correlated with the development of endometrial cancer [7], and insulin increases the proliferation of endometrial cancer cells *in vitro* [8]. Further, raised insulin levels may be associated with breast cancer [9] and ovarian cancer [10]. It has also been proposed that high levels of IGF-I are associated with an increased risk of several cancers, including endometrial cancer [11,12], and a paracrine role for IGFs in tumour development has been suggested [13]. In addition, IGF is associated with enhanced cell proliferation.

Adiponectin is synthesised and secreted into the peripheral circulation only by adipose tissue. Evidence suggests that non-obese women have a highly circulated level of adiponectin hormone relative to obese women, suggesting the physiological role of adiponectin is the opposite of leptin [14]. Furthermore, women with high adiponectin levels are at a lower risk of endometrial cancer than subjects with low plasma concentrations of adiponectin [12]. Thus, adiponectin has been associated negatively with endometrial cancer development [15,16]. Additionally, there was an inverse association between circulating adiponectin levels *in vivo* and the incidence of malignancies linked to obesity, including breast cancer [17] and ovarian cancer [10,18].

Cancer cell culture *in vitro* routinely uses a chemically synthesised mixture of vitamins, minerals and fetal bovine serum as a primary source of hormones and growth factors. However, this practice does not include various concentrations of specific hormones concurrently present as a cocktail mixture at physiological levels. Therefore, the results from such an experiment provide unrealistic information irrelevant to *in vivo* conditions.

In this study, we investigated the effects of hormones on two endometrial cell lines (Ishikawa and HEC-1A), an ER-PR receptor-positive breast cell line (MCF-7), and an ovarian cancer cell line (SKOV-3). We hypothesised that responding to obesity-related hormones was cell line dependent, and endometrial cancer cell lines shall have a stark response to the hormone compared to breast and ovarian cancer cell lines.

Materials and Methods

Cell culture

Cells were maintained in medium 199 (M199) with 10% FBS (Thermo Fisher Scientific, Auckland, New Zealand) in a culture flask. Cells were at approximately 70% confluence in 37°C and 5%/95% CO_2/air incubator before adding treatments and collection. Approximately 50,000 cells/well were seeded in 24 well flat bottom plates supplemented with an M199 medium with 10% FBS (1 ml). Cells were incubated for 48 hr to allow cells to attach, and then the medium was discarded and replaced with M199 without FBS (1 ml) to minimise the effects of remnant hormones and growth factors from FBS. After 24 hr, the medium was removed from cell culture wells, and 1 mL of serum-free medium supplemented with selected concentrations of hormones was placed in each well. The concentrations of Leptin (50 ng/mL), adiponectin (20 and 100 ng/mL), insulin (580 ng/mL; 100 nM), and insulin-like growth factor-1 (100 ng/mL) were chosen for cell studies. All hormones were purchased from GenWay, USA. Leptin (Recombinant human Leptin, Cat. No, 10-783-313250). Adiponectin (Adiponectin recombinant human, Cat. No, 10-002-38001). Insulin (Insulin human, Cat. No, 10-663-45749), and insulin-like growth factor-1 (IGF-I) (Recombinant human IGF-I, Cat. No, CR1500C).

Immunofluorescence microscopy

Round coverslips with 18 mm diameter were sterilised in 70% ethanol solution and used to culture cells for immunofluorescent imaging. The sterile round coverslips were placed in 12 well plates. Then the medium with 10% FBS containing cancer cell lines were added to each well and was incubated for 48 hr to allow adherence and proliferation. After that, the medium was removed, and the cells on the coverslip were fixed with 4% paraformaldehyde in PBS, pH 7.4, for 45 min at room temperature. The cells were washed with cold PBS and permeabilised with cold methanol (95%). Cells were incubated with the primary antibody in 2.5% BSA in PBS at 4°C overnight. Incubation in the dark was performed with a secondary antibody conjugated with fluorescent molecule Atto 594 nm (Sigma-Aldrich, Cat. No, 08717, USA). Alexa 488 Phalloidin (CytoPainter Phalloidin iFluor 488 reagents, Cat. No, ab176753, USA) was added to detect actin. Negative controls without primary or secondary antibodies were performed, and specificity absorption controls were performed for the two cases (insulin receptor and adiponectin receptor) where it was available. The cells were covered with aluminium foil to protect them from light. Cells were incubated with Hoescht (20 µg/mL) for 20 minutes at room temperature in the dark, and cells were washed with a mixture of cold PBS and 0.05% Tween-20. The anti-fading solution was added to the cells on a coverslip. Localisation of immunostaining was visualised with an epifluorescent microscope (Carl Zeiss, Imager.Z1, Germany). Confocal images were collected using the ApoTome software, and 3D images were generated using the ZEN application software (Carl Zeiss, Germany).

Western blotting

The conditioned medium was removed, and then cells were washed with 0.5 mL PBS. Then cells were lysed with 100 µL RIPA lysis buffer. The lysis buffer contained 50 mM Tris base (2- Amino-2-(hydroxymethyl)-1,3-propanediol, Roche, Cat. No, 708976, USA), 150 mM NaCl (MERCK, Cat. No, K 33591704506, Germany), 5 mM ethylenediaminetetraacetic acid, 0.1% w/vol SDS (sodium dodecyl sulfate, BDH, Cat. No, 44244, UK), 1% vol/vol) NP-40 (Acetyl phenol ethylene oxide, SIGMA Aldrich, Cat. No, N-6507, USA), 0.5% w/vol sodium deoxycholate, 1 mM NaVO4, 10% glycerol (SIGMA Aldrich, Cat. No, G 6279-1 litre, Malaysia), and 1 protease inhibitor cocktail tablets (Complete Mini, Roche, New Zealand), pH 7.4). The total protein was measured by Bio-Rad DC protein assay kit (Cat. 500-0113, 500-0114 and 500-0115, CA, USA). Sample buffer (0.2% (vol/vol) bromophenol blue, 25% (vol/vol) glycerol, 10% SDS in Tris-HCl, and pH 6.8) was added, and protein lysates were boiled for 10 minutes. Before loading, the cell lysates were mixed and centrifuged at 9,700g for 5 minutes. A total of 10 µg protein lysate was loaded and separated by SDS-PAGE using a 7% stacking gel and a 10% separating gel.

The SDS-PAGE was run at 120 volts using a Tris-glycine running buffer. The SDS-PAGE markers used were MagicMark™ XP Western Standard (Thermo Fisher Scientific, New Zealand) and Precision Plus Protein standard (Bio-Rad, Hercules, USA). Proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (BioRad, Cat. No, 162-0177, USA) in ice-cold transfer buffer (25 mmol/l Tris base, 200 mmol/l glycerin, MiliQ water) at 100 V for 60 minutes. Specific immuno-detection was carried out by incubation with relevant primary antibodies. Antigens were revealed using the ECL developing solution (Amersham, ECL Prime Western Blotting Detection Reagent, ge- 9466588; GE Healthcare, UK). The protein bands were visualised, and densitometry analysis was performed using Alliance 4.7, Unitec (Cambridge, UK). Cell lysates were collected from at least three independent cell culture experiments. The primary antibodies used in this study were anti-AdipoR1 (sc-99183), anti-AdipoR2 (sc-99184), anti-Ob-R (sc- 8325), anti- InsR (sc-711) and anti-IGFR (sc-7952). The two secondary antibodies used in this study were bovine anti-rabbit IgG-HRP (sc-2385) and bovine anti-mouse IgG-HRP (sc-2380).

Detection of apoptosis

The cells were seeded in 24-well culture plates at a density of 5×10^4 cells/well in the M199 medium supplemented with 10% FBS as a control medium or with hormone treatments for 48 hr. Cells were detached and collected in 1.5 mL tubes. Briefly, after centrifugation for 10 minutes at 2,000 rpm, the cell pellets were re-suspended in the Annexin assay buffer (200 µL). The buffer contained 10 mM PIPES, 140 mM NaCl, and 2.5 mM $CaCl_2$ (all from Sigma-Aldrich) and was adjusted to pH 7.4. A 5 µL of Annexin-FITC was added to the Eppendorf tube to detect apoptotic cells. The re-suspended cell pellets were kept in the buffer for 20 minutes at room temperature. Cells were then incubated with 1.3 µL of propidium iodide (PI) for 10 minutes in the dark at room temperature to detect necrotic cells. Then Annexin

assay buffer (200 µL) was added again. Finally, 200 µL of cell suspension was added to the 96-well plate in duplicate. Samples were then analysed in a Cytomics FC500 MPL flow cytometer (Beckman Coulter, Brea, CA, USA).

VEGF ELISA assay

Conditioned media from control and treated cells were collected and centrifuged to remove debris. ELISA analysis was performed according to the manufacturer's instructions (VEGF ELISA kit; DuoSet ELISA Development R&D System human VEGF, Minneapolis, MN, USA). The kit components consisted of Capture Antibody, Detection Antibody, Standard, Stop solution, Streptavidin-HRP, and Substrate solution. The cell numbers for corresponding cultures were counted, and the level of VEGF that was secreted was determined per $1x10^6$ cells. The absorbance (A) values were measured at a wavelength of 450 nm. The standard curve was plotted by Prism statistical software. The experiments were performed with six replicates, and the experiment was performed three times.

Statistical analysis

Data were expressed as mean ± SEM from at least three independent experiments performed in triplicate. Statistical analyses were performed using GraphPad Prism 7. Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) multiple range test or logarithmic analysis and Student's t-test or one-way analysis of variance (ANOVA) as appropriate. A p-value of < 0.05 was considered to indicate a significant difference between parameters. Each experiment was repeated at least three times in duplicate.

Results

With immunohistochemistry and western blotting we established that the four cell lines possessed receptors for the four hormones being studied (Figure 1).

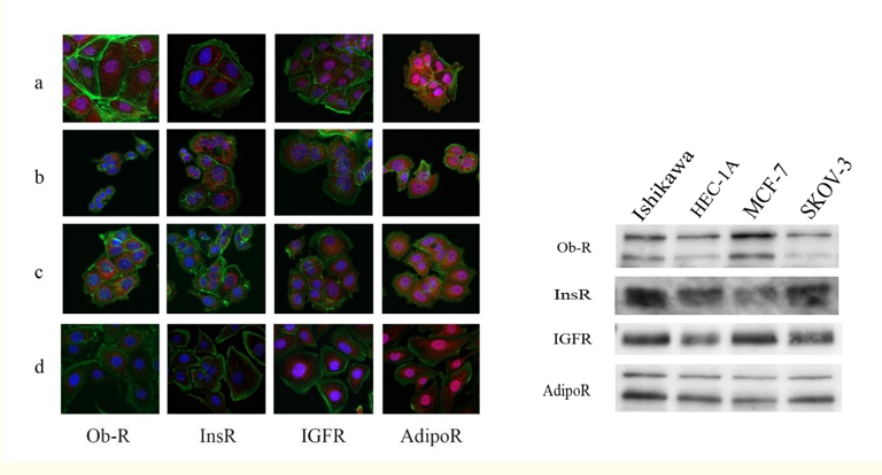

Figure 1: *Left. Images of (a) Ishikawa endometrial, (b) HEC-1A endometrial, (c) MCF-7 breast and (d) SKOV-3 ovarian cancer cell lines. Images exhibit receptors for Leptin (Ob-R), insulin (InsR), insulin-like growth factor-1 (IGF-1R) and adiponectin (AdipoR) receptors. Red receptors, blue cell nuclei and green actin. Right. Immunodetection bands of Western blots.*

Ishikawa cells were observed to respond to the obesity-related hormones (Figure 2). With leptin, insulin, and IGF-I, cell numbers increased significantly ($p < 0.001$) (Figure 2a). Interestingly, low concentrations of adiponectin at 20 ng/mL significantly ($p < 0.001$) increased cell number of the Ishikiwa line relative to the control. However, adiponectin at 100 ng/mL significantly ($p < 0.05$) reduced

cell numbers compared to controls. All hormones tended to induce apoptotic cells (Figure 2b), but results were not statistically different, except adiponectin at 100 ng/mL ($p < 0.05$). Leptin ($p < 0.05$), insulin ($p < 0.001$), and IGF-1 ($p < 0.05$) significantly reduced the secretion of VEGF in cell-conditioned media (Figure 2c). However, both concentrations of adiponectin significantly ($p < 0.001$) increased the secretion of VEGF.

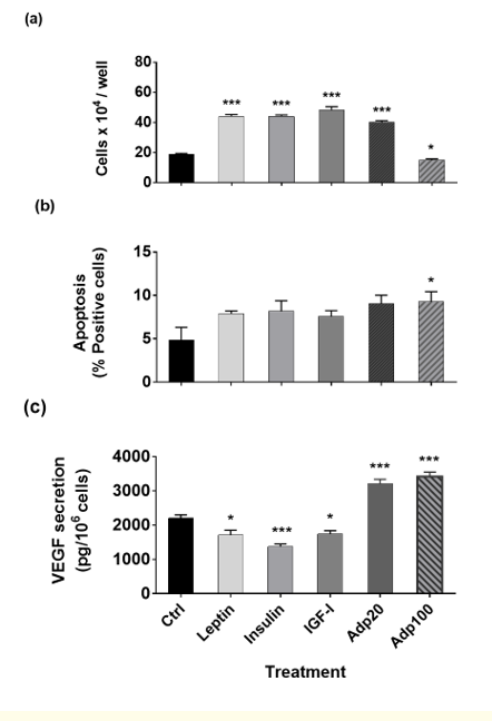

Figure 2: *(a) Cell numbers, (b) apoptosis and (c) VEGF secretion from the Ishikawa cell line treated with obesity-related hormones. Leptin (50 ng/mL), insulin (580 ng/mL), IGF-I (100 ng/mL), and adiponectin at 20 ng/mL (Adp20) and 100 ng/mL (Adp100) stimulated cells. Data are expressed as means ± SEM, n = 9. *, P < 0.05; ***, P < 0.001 compared to control.*

Leptin, insulin and IGF-I significantly ($p < 0.001$) promoted increased cell number (Figure 3a). However, both concentrations of adiponectin significantly ($p < 0.05$, 0.001) reduced cell numbers. None of the hormones modified the apoptotic levels in HEC-1A endometrial cells (Figure 3b). Leptin significantly reduced VEGF secretion ($p < 0.05$), insulin ($p < 0.01$), and IGF-1 ($p < 0.01$) in the HEC-1A cell line (Figure 3c). Again, similar to Ishikawa cells, both adiponectin concentrations significantly ($p<0.05$) increased the secretion of VEGF.

Only leptin and IGF-1 hormones significantly ($p < 0.01$) promoted cell growth in the MCF-7 cell line (Figure 4a). Interestingly, all hormones significantly increased apoptotic cells (Figure 4b). Only leptin significantly ($p < 0.01$) decreased VEGF secretion to the cell culture medium (Figure 4c). On the contrary, insulin and IGF-1 significantly ($p < 0.01$) increased the secretion of VEGF. Adiponectin did not alter the secretion of VEGF in the MCF-7 cell line.

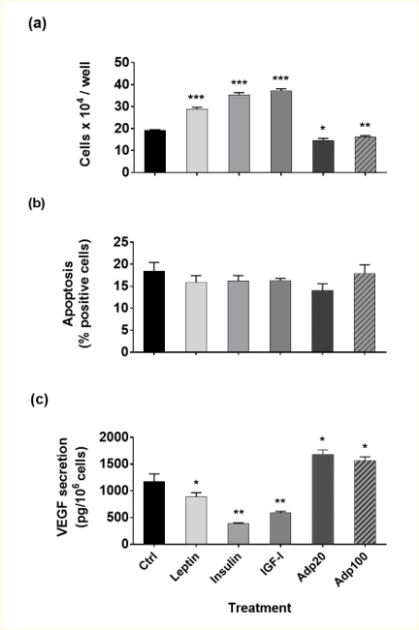

Figure 3: *(a) Cell numbers, (b) apoptosis and (c) VEGF secretion from HEC-1A cells treated with obesity-related hormones. Leptin (50 ng/mL), insulin (580 ng/mL), IGF-I (100 ng/mL), and adiponectin at 20 ng/mL (Adp20) and 100 ng/mL (Adp100) stimulated cells. Data are expressed as means ± SEM, n = 9. *, p < 0.05; **p < 0.01; ***, p < 0.001 compared to control.*

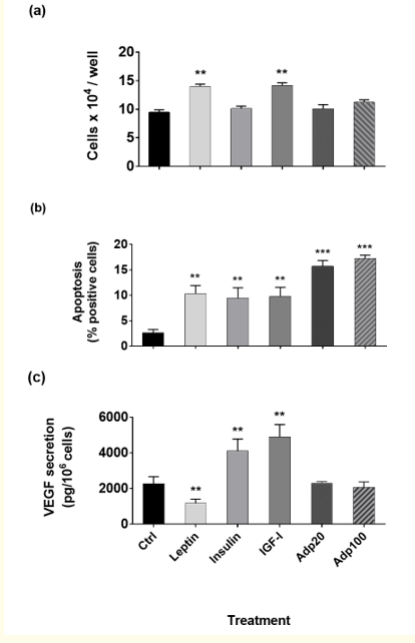

Figure 4: *(a) Cell numbers, (b) apoptosis and (c) VEGF secretion from the breast cancer cell line (MCF-7) treated with obesity-related hormones. Leptin (50 ng/mL), insulin (580 ng/mL), IGF-I (100 ng/mL), and adiponectin at 20 ng/mL (Adp20) and 100 ng/mL (Adp100) stimulated cells. Data are expressed as means ± SEM, n = 9. *, p < 0.05; **p < 0.01; ***, p < 0.001 compared to control.*

SKOV-3 exhibited a cell proliferation pattern markedly distinct from Ishikawa, HEC-1A, or MCF-7. Only a marked increase in cell number by adiponectin 100 ng/mL was observed in the SKOV-3 cell line (Figure 5a). There was no change in apoptosis of hormone-stimulated cells (Figure 5b). Secretion of VEGF was significantly increased ($p < 0.001$) in insulin-stimulated SKOV-3 cells (Figure 5c). IGF-1 also notably increased VEGF secretion ($p < 0.5$). Adiponectin significantly reduced VEGF secretion at 100 ng/mL ($p < 0.05$).

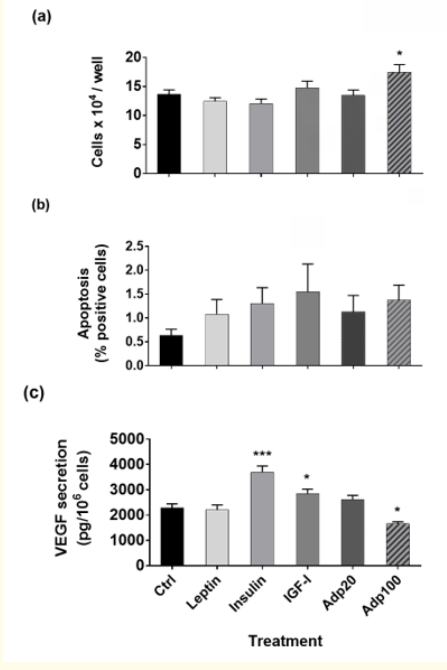

Figure 5: *(a) Cell numbers, (b) apoptosis and (c) VEGF secretion from ovarian cancer cell line (SKOV-3) cells treated with obesity-related hormones. Leptin (50 ng/mL), insulin (580 ng/mL), IGF-I (100 ng/mL), and adiponectin at 20 ng/mL (Adp20) and 100 ng/mL (Adp100) stimulated cells. Data are expressed as means ± SEM, n = 9. *, $p < 0.05$; **$p < 0.01$; ***, $p < 0.001$ compared to control.*

Discussion and Conclusion

This study presented the effects on cell proliferation, apoptosis, and the secretion of VEGF of leptin, insulin and IGF-I, which are considered tumorigenic, and of two concentrations of adiponectin, which is considered an anti-tumorigenic hormone. The model at the centre of this study is the endometrial cancer cell lines. The Ishikawa cell line was initially isolated from a woman with low-grade type I endometrial cancer, the most common subtype of endometrial cancer. The Ishikawa cell line has become the most widespread human endometrial-derived cell culture model because it has oestrogen [19], progesterone [20] and androgen receptors [21]. Furthermore, Ishikawa cells have a PTEN mutation, a gene mutation commonly found with type I endometrial cancer. One additional endometrial cancer cell line, HEC-1A, has a PTEN wild type and is used to compare with the Ishikawa cell line. In addition, cancer cell lines from breast origin, MCF-7, and ovarian clear cell subtype, SKOV-3, were included in this study.

All cell lines have a detectable expression of protein receptors for leptin, insulin, IGF-1 and adiponectin ligands. Interestingly, all receptors are found in the cytoplasm, plasma membrane and nucleus. Localisation of these receptors is consistent with reports of clinical endometrial cancer tissue [22-24]. Therefore, we postulated that all cell lines should similarly respond to hormones. However, this is not the case. Instead, it seems to be that endometrial cancer cell lines increased cell growth with leptin, insulin and IGF-1. Paradoxically, at

a physiologically low concentration, adiponectin can also increase cell growth of the Ishikawa line but did not produce a similar effect in the HEC-1a cells. The mutation status of the PTEN gene, leading to a loss function of the PTEN protein, might contribute to this distinct response to the low concentration of adiponectin, but more endometrial cancer cell lines need to be used to firmly confirm this conclusion.

Adiponectin at a physiologically high concentration suppressed cell growth in both endometrial cancer cell lines, consistent with previous reports [25,26]. Thus, our study discovers adiponectin properties in endometrial cancer associated with tumorigenic activity at a low physiologically relevant concentration. We, however, do not have any scientific rationale to explain this growth promotion activity of adiponectin in this manuscript. Nevertheless, it is possible to form the hypothesis that obese cancer patients with high leptin and low adiponectin could facilitate aggressive tumour growth compared to patients with high leptin and high adiponectin. Collectively, our findings would highlight the importance of low adiponectin in the microenvironment of endometrial tumorigenic steps.

Adiponectin concentrations significantly increased VEGF secretion in endometrial cancer cell lines, but no such effect was observed in breast or ovarian cancer cell lines. The underlying cellular mechanism by which adiponectin regulates VEGF may involve the activation of nutrient sensory pathways, potentially through the activation of AMPK protein [27-29]. This activation may induce a cellular state resembling nutrient deficiency, leading to the upregulation of VEGF expression and secretion. The recruitment of the vascular system around the tumour in response to elevated VEGF levels could enhance nutrient supply to the tumour. It is worth noting that leptin, insulin and IGF-1 hormone significantly reduced VEGF secretion, but we could not rule out that the overall intracellular level of VEGF in these hormone-stimulated endometrial cancer cells would change. Leptin, insulin and IGF-1 may not stimulate the activation of AMPK similarly to adiponectin.

The effects of hormones on VEGF secretion in cancer cells have not been well defined. This aspect is vital to understand how angiogenic support maintains the growth of solid tumours. Thus, the three putatively tumorigenic hormones (leptin, insulin and IGF-I) had similar effects on VEGF secretion despite selective effects on cell number growth that were observed. The results indicate that the factors do not have a uniform mechanism of action on all oncogenic processes (such as proliferation and VEGF-induced angiogenesis).

The findings from this study indicate that the four obesity-related hormones examined-leptin, insulin, IGF-I, and adiponectin-have the potential to directly contribute to cancer development as endocrinological factors. However, the specific effects of these hormones vary depending on the tissue type and, potentially, the underlying driving mutation. Elaborating on the intricate network connecting hormones, cancer cells, and the tumour microenvironment in an *in vivo* setting holds significant clinical implications. By delving deeper into these relationships, valuable insights can be gained for the development of targeted therapeutic strategies and improved patient outcomes.

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Conflict of Interest

We declare there is no financial interest or any conflict of interest.

Bibliography

1. Trayhurn and JH Beattie. "Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ". *Proceedings of the Nutrition Society* 60.3 (2001): 329-339.

2. Pedone C., *et al*. "Longitudinal association between serum leptin concentration and glomerular filtration rate in humans". *PLoS One* 10.2 (2015): e0117828.

3. Villavicencio A., *et al*. "The effect of overweight and obesity on proliferation and activation of AKT and ERK in human endometria". *Gynecologic Oncology* 117.1 (2010): 96-102.

4. Ashizawa N., *et al*. "Serum leptin-adiponectin ratio and endometrial cancer risk in postmenopausal female subjects". *Gynecologic Oncology* 119.1 (2010): 65-69.

5. Sharma D., *et al*. "Leptin promotes the proliferative response and invasiveness in human endometrial cancer cells by activating multiple signaltransduction pathways". *Endocrine-Related Cancer* 13.2 (2006): 629-640.

6. Cusi K., *et al*. "Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle". *Journal of Clinical Investigation* 105.3 (2000): 311-320.

7. Gunter MJ., *et al*. "A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer". *Cancer Epidemiology, Biomarkers and Prevention* 17.4 (2008): 921-929.

8. Guo S., *et al*. "Oncogenic role and therapeutic target of leptin signaling in breast cancer and cancer stem cells". *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* 1825.2 (2012): 207-222.

9. Rose D and L Vona-Davis. "The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression". *Endocrine-Related Cancer* 19.6 (2012): R225-R241.

10. Otokozawa S., *et al*. "Associations of Serum Isoflavone, Adiponectin and Insulin Levels with Risk for Epithelial Ovarian Cancer: Results of a Case-control Study". *Asian Pacific Journal of Cancer Prevention: APJCP* 16.12 (2014): 4987-4991.

11. Bruchim I., *et al*. "The IGF Hormonal Network in Endometrial Cancer: Functions, Regulation, and Targeting Approaches". *Frontiers in Endocrinology* 5 (2014): 76.

12. Mantzoros C., *et al*. "Adiponectin and breast cancer risk". *The Journal of Clinical Endocrinology and Metabolism* 89.3 (2004): 1102-1107.

13. Samani AA., *et al*. "The role of the IGF system in cancer growth and metastasis: overview and recent insights". *Endocrine Reviews* 28.1 (2007): 20-47.

14. Arita Y., *et al*. "Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity,1999". *Biochemical and Biophysical Research Communications* 425.3 (2012): 560-564.

15. Cong L., *et al*. "Human adiponectin inhibits cell growth and induces apoptosis in human endometrial carcinoma cells, HEC-1-A and RL95 2". *Endocrine-Related Cancer* 14.3 (2007): 713-720.

16. Cust AE., *et al*. "Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women". *The Journal of Clinical Endocrinology and Metabolism* 92.1 (2007): 255-263.

17. Korner A., *et al*. "Total and high-molecular-weight adiponectin in breast cancer: in vitro and in vivo studies". *The Journal of Clinical Endocrinology and Metabolism* 92.3 (2007): 1041-1048.

18. Jin JH., *et al*. "Association of plasma adiponectin and leptin levels with the development and progression of ovarian cancer". *Obstetrics and Gynecology Science* 59.4 (2016): 279-285.

19. Holinka CF., *et al*. "Responses to estradiol in a human endometrial adenocarcinoma cell line (Ishikawa)". *The Journal of Steroid Biochemistry* 24.1 (1986): 85-89.

20. Lessey BA., *et al*. "Characterization of the functional progesterone receptor in an endometrial adenocarcinoma cell line (Ishikawa): progesterone-induced expression of the alpha1 integrin". *The Journal of Steroid Biochemistry and Molecular Biology* 59.1 (1996): 31-39.

21. Lovely LP., *et al*. "Characterization of androgen receptors in a well-differentiated endometrial adenocarcinoma cell line (Ishikawa)". *The Journal of Steroid Biochemistry and Molecular Biology* 74.4 (2000): 235-241.

22. Yabushita H., *et al*. "Clinicopathological roles of adiponectin and leptin receptors in endometrial carcinoma". *Oncology Letters* 7.4 (2014): 1109-1117.

23. Kaplan SA. "The insulin receptor". *The Journal of Pediatrics* 104.3 (1984): 327-336.

24. Weigang B., *et al*. "Immunohistochemical localization of insulin-like growth factor 1 receptors in benign and malignant tissues of the female genital tract". *Tumor Biology* 15.4 (1994): 236-246.

25. Jarde T., *et al*. "Molecular mechanisms of leptin and adiponectin in breast cancer". *European Journal of Cancer* 47.1 (2011): 33-43.

26. Gallagher EJ and D LeRoith. "Epidemiology and molecular mechanisms tying obesity, diabetes, and the metabolic syndrome with cancer". *Diabetes Care* 36.2 (2013): S233-S239.

27. Chunga SJ., *et al*. "ADIPOQ/adiponectin induces cytotoxic autophagy in breast cancer cells through STK11/LKB1-mediated activation of the AMPK-ULK1 axis". *Autophagy* 13.8 (2017): 1386-1403.

28. Li Y., *et al*. "Dual Roles of the AMP-Activated Protein Kinase Pathway in Angiogenesis". *Cells* 8 (2019): 752.

29. Pham D., *et al*. "Globular Adiponectin Inhibits Breast Cancer Cell Growth through Modulation of Inflammasome Activation: Critical Role of Sestrin2 and AMPK Signaling". *Cancers* 12.613 (2020).

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