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Molecules and Cells

Visfatin Stimulates Proliferation of MCF-7 Human Breast Cancer Cells

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Obesity, a condition characterized by increased fat content and altered secretion of adipokines, is a risk factor for postmenopausal breast cancer. Visfatin has recently been established as a novel adipokine that is highly enriched in visceral fat. Here we report that visfatin regulated proliferation of MCF-7 human breast cancer cells. Exogenous administration of recombinant visfatin increased cell proliferation and DNA synthesis rate in MCF-7 cells. Furthermore, visfatin activated G1-S phase cell cycle progression by upregulation of cyclin D1 and cdk2 expression. Visfatin also increased the expression of matrix metalloproteinases 2, matrix metalloproteinases 9, and vascular endothelial growth factor genes, suggesting that it may function in metastasis and angiogenesis of breast cancer. Taken together, these findings suggest that visfatin plays an important role in breast cancer progression.

INTRODUCTION

Obesity and related metabolic alterations are associated with increased risk of cancer, especially breast cancer (Stephenson and Rose, 2003). Body mass index (BMI) has been found to be related to the risk of breast cancer in postmenopausal women (Loi et al., 2005; Porter et al., 2006). In addition, obese breast cancer patients are likely to have worse outcomes than non-obese patients. Obesity is closely linked to an increase in energy storage in the form of triglycerides in adipose tissue. Adipocytes secrete a number of different factors that are commonly referred to as 'adipokines' (adipocyte-derived cytokines). Adipokines are involved in a variety of biological functions, including the regulation of energy balance, glucose homeostasis, lipid metabolism, and inflammation (Karastergiou and Mohamed-Ali, 2010).

Recently, there has been considerable interest in the role of adipokines in breast carcinogenesis. Although several adipokines, including leptin, resistin, and adiponectin, are closely related to breast cancer progression (Kang et al., 2007; Vona-Davis and Rose, 2007), the mechanism by which adipokines influence this process is unclear. Visfatin, a novel adipocyte-derived adipokine, is highly enriched in the visceral fat of both humans and mice, and plasma levels of visfatin increase during the development of obesity (Fukuhara et al., 2005). Visfatin is also known as pre-B cell enhancing factor (PBEF), a growth factor for early B cell proliferation (Samal et al., 1994), and it is the secretory form of nicotinamide phosphoribosyl-transferase (Nampt) (Imai, 2009), the rate-limiting enzyme of mammalian nicotinamide adenine dinucleotide (NAD) biosynthesis. Visfatin plays an important role in insulin secretion from pancreatic beta cells and affects a variety of metabolic and stress responses (Garten et al., 2009; Revollo et al., 2007). Recent studies have shown that circulating visfatin levels are increased in the serum of gastric cancer patients (Nakajima et al., 2009) and that the proliferation of prostate cancer cells is increased by exogenous treatment with visfatin (Patel et al., 2010). In breast cancer cells, transcription of human visfatin genes is regulated by hypoxia-inducible factor-1, a key factor in malignant tumor progression (Bae et al., 2006).

The relationship between visfatin levels and breast cancer risk is unclear, and the molecular basis for such a link remains poorly understood. To clarify the relationship between visfatin and breast cancer development, we therefore investigated whether visfatin regulated the proliferation of MCF-7 breast cancer cells.

MATERIALS AND METHODS

Cell culture and treatment

The MCF-7 human breast cancer cell line was obtained from ATCC (USA) and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, streptomycin (0.1 mg/ml), and penicillin (100 U/ml) at 37°C under a 5% CO_2 atmosphere. Cells were seeded in 96- or 6-well culture plates and allowed to attach overnight in an incubator. The complete medium was then replaced with serum-free medium for 24 h to allow for cell cycle synchronization. Cells were then treated with visfatin (Adipogen, Korea) and incubated for 24 h and 48 h, at which times cell proliferation assays were performed. For gene expression assays, cells were seeded at 2×10^5 cells/well in 6-well plates and incubated overnight. The total medium was then

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replaced with serum-free medium for 24 h to allow for cell cycle synchronization. Cells were treated with visfatin and incubated for 6 h.

Cell proliferation assay

Growing cells (5×10^3 cells/well) were seeded in 96-well plates and incubated with various concentrations of recombinant human visfatin for 24 h and 48 h. Cell number and viability were determined using a hemocytometer after staining with trypan blue. Cell proliferation was analyzed using the MTS assay kit (Promega, USA) according to the manufacturer's instructions. Briefly, 20 μl of Cell Titer 96®AQueous One Solution Reagent containing tetrazolium compound [3-(4,5-di-methylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine methosulfate; PMS) was added per well, and the cells were incubated for one hour at 37°C in a humidified, 5% CO_2 atmosphere. Formazan production by viable cells was measured at 490 nm with a 96-well plate reader (Wallac victor 1420 multilabel counter, Finland).

Real-time PCR

Total RNA was isolated from the cultured cells using Trizol reagent (Sigma, USA). Total RNA (2 μg) was reverse-transcribed and amplified by real-time PCR using several sets of primers: Cyclin D1 sense primer, 5′-GAA CTA CCT GGA CCG CTT CC-3′; antisense primer, 5′-GAG CTT GTT CAC CAG GAG CA-3′; cdk2 sense primer, 5′-AGA AAA TCC GCC TGG ACA CT-3′; antisense primer, 5′-GAG AGC AGA GGC ATC CAT GA-3′; MMP2 sense primer, 5′-TGG CAA GTA CGG CTT CTG TC-3′; antisense primer, 5′-TTC TTG TCG CGG TCG TAG TC-3′; MMP9 sense primer, 5′-TGC GCT ACC ACC TCG AAC TT-3′; antisense primer, 5′-GAT GCC ATT CAC GTC GTC CT-3′; VEGF sense primer, 5′-ATC TTC AAG CCA TCC TGT GTG C-3′; antisense primer, 5′-TGC GCT TGT CAC ATT TTT CTT G-3′. Real-time PCR reactions [20 μl total volume, containing 5 pmol primer, 10 μl SYBR green dye (Qiagen, USA) and 2 μl cDNA] were performed using the DNA Engine Opticon Continuous Fluorescence Detection System (MJ Research Inc., USA) for approximately 40 cycles.

Bromodeoxyuridine (BrdU) incorporation assay and immunofluorescence

The cells were seeded on cover slips. After 24 h of serum-starved incubation, visfatin (500 ng/ml) was added to the serum-free medium and the cells were incubated for an additional 24 h. After visfatin treatment, the cells were incubated with 20 μM 5-bromo-2-deoxyuridine (BrdU; Sigma) for 45 min, fixed with 70% ethanol, and the DNA was denatured with 2 N HCl. Fixed slides were washed with 0.1 M phosphate buffer (PB) for 30 min, and incubated with blocking buffer (4% nonfat milk, 0.4% Triton X-100, 0.1 M PB). After washing the slides three times with 0.1 M PB for 5 min, slides were incubated with mouse monoclonal anti-BrdU antibody (Abcam, USA) for 2 h at room temperature and washed three times for 10 min. The sections were incubated with biotin-conjugated donkey anti-mouse IgG (Vector Labs, USA; 1:400) in PB for 2 h. Staining was performed using an ABC kit (Vector Labs) and color development was achieved by incubating the sections for 5 min with the tyramide signal amplification system (NEN Life Science, USA). For each coverslip, 3,000 cells were counted and the percentage of BrdU-positive cells was determined.

Flow cytometry for cell cycle analysis

Cells were plated at a density of approximately 2×10^5 cells in 6 well culture plates and allowed to grow for 24 h. The complete medium was replaced with serum-free medium for 18 h for cell cycle synchronization. Cells were then treated with visfatin and incubated for 24 h. At this time cells were harvested by trypsinization and subsequently processed for flow cytometric analysis. In brief, cells were washed twice in chilled PBS and fixed in 70% ethanol on ice. After RNase A (200 μg/ml) treatment for 30 min at 37°C, 50 μg/ml propidium iodide (PI) was added to the cell pellet and incubated in the dark for 30 min on ice. The fluorescence of PI was collected through a 585 nm filter in FACScan flowcytometer (BD Biosciences, USA).

Statistical analysis

Differences among groups were analyzed using one-way ANOVA with Dunnett's multiple comparison test. Student's *t*-test was used for comparisons between two groups.

Visfatin stimulates proliferation of MCF-7 cells

To examine the effect of visfatin on proliferation of breast cancer cells, MCF-7 cells were incubated in serum-free medium supplemented with recombinant human visfatin for 24 and 48 h at various concentrations. Cell viability was analyzed using the MTS assay. Viability of MCF-7 cells increased in a dose-dependent manner after administration of visfatin, and the effect of visfatin reached a peak at 1 μg/ml (Fig. 1A). To further confirm the effect of visfatin on proliferation of breast cancer cells, we counted the number of viable MCF-7 cells after plating and treatment with visfatin in the same manner as during the MTS assay. Control and visfatin-treated cells were compared for the number of live cells under serum-free conditions using a trypan blue exclusion assay. Cell number was greater in the visfatin-treated wells than in the control wells (Fig. 1B). As shown in Fig. 1C, visfatin-treated MCF-7 cells more rapidly proliferate than control MCF-7 cells. Doubling time of visfatin treated-MCF-7 was about 18 h compared to about 24 h for control MCF-7 cells.

Visfatin stimulates BrdU incorporation

To determine the potential effect of visfatin on breast carcinogenesis, we evaluated the incorporation of BrdU as an indicator of DNA synthesis rate in MCF-7 cells that had been treated with visfatin in serum-free medium. Fluorescence microscopy analyses showed a higher percentage of BrdU-stained cells among the visfatin-treated cells than the vehicle-treated control cells (Fig. 2). These data indicated that stimulation of proliferation by exogenous treatment of MCF-7 cells with visfatin was closely related to increased DNA synthesis.

Visfatin upregulates levels of cyclin D1 and cdk2 mRNA and proportion of S phase

Abnormal cell cycle is a common feature of cancer, which frequently affects cyclin-dependent kinases (cdks) and their regulator, cyclin D1 (Sherr, 1994). To clarify whether visfatin stimulates proliferation of MCF-7 cells by affecting cell cycle progression, we examined expression patterns of cyclin D1 and cdk2, two genes that are upregulated at the S phase cell cycle checkpoint. We found that exogenous visfatin treatment of MCF-7 cells increased levels of cyclin D1 and cdk2 mRNA (Fig. 3). To further confirm effect of visfatin in the regulation of cell cycle, we investigated cell cycle proportion by flow cytometry. The cell cycle analysis revealed that visfatin induced a significant decrease of G1, but an increased S phase of the cells within 24 h of normal growth conditions. These data suggested that visfatin may increase of cell proliferation through acceleration of the cell cycle.

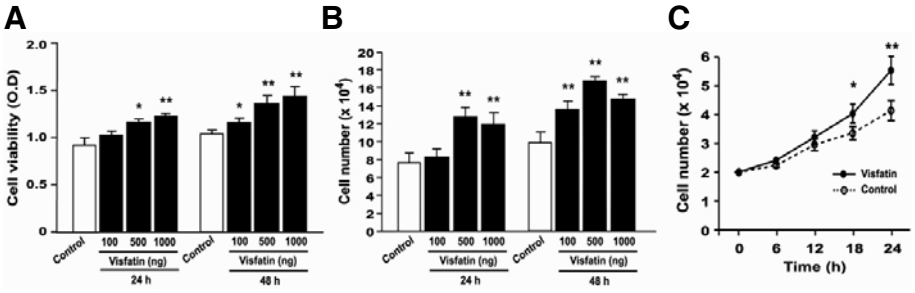

Fig. 1. Effect of visfatin on cell proliferation in MCF-7 cells. MCF-7 cells were plated in 96-well plates and treated with visfatin at the indicated concentrations, followed by cell viability testing using the MTS assay and live cell counting. (A) MCF-7 cell viability determined by absorbance at O.D 490 nm was increased by visfatin treatment. (B) MCF-7 viable cell number was also increased by visfatin treatment. (C) After visfatin treatment, cell number was counted every 6 h intervals for 24 h to determine proliferation rate. Doubling time of visfatin-treated MCF-7 cells was significantly shorter than that of vehicle-treated MCF-7 cells. All data were expressed as the mean ± S.E.M. of at least six wells per group. *, $p < 0.05$; **, $p < 0.01$ for visfatin vs. control.

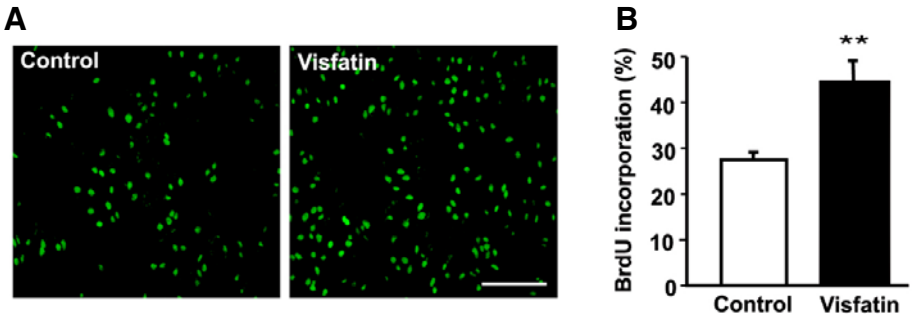

Fig. 2. Effect of visfatin on BrdU incorporation in MCF-7 cells. Cells were cultured with visfatin (500 ng/ml) for 24 h and stained with an antibody against BrdU. (A) Representative photographs of BrdU-positive cells in each group. (B) At least 500 cells were counted in each experiment, and the percentage of BrdU-positive cells was calculated and compared to the control. Data are expressed as the mean ± S.E.M. of at least six slides per group. **, $p < 0.01$ for visfatin vs. control.

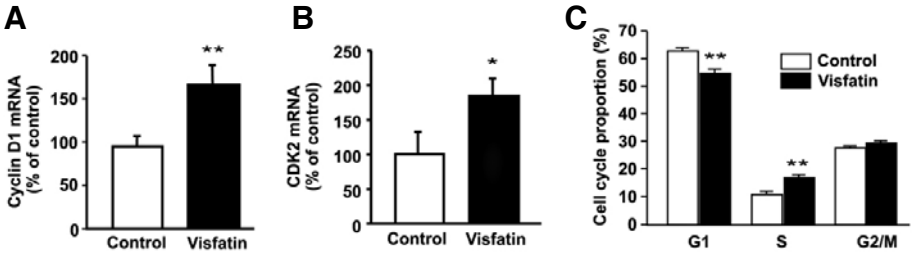

Fig. 3. Effect of visfatin on cyclin D1 and cdk2 gene expression and proportion of cell cycle. MCF-7 cells were plated in 6-well plates and then incubated for 6 h and 24 h in the presence of visfatin (500 ng/ml) to examine cyclin D1 and cdk2 gene expression by real-time PCR and cell cycle proportion by FACS assay. Treatment of MCF-7 cells with visfatin resulted in increased cyclin D1 (A) and cdk2 (B) mRNA levels. (C) Distribution of cell cycle was determined and expressed as percentage of cells in any one phase. The bars represent relative proportion of cells in G1, S, and G2/M phases, respectively. Data are represented as the mean ± S.E.M (n = 6). *, $p < 0.05$; **, $p < 0.01$ for visfatin vs. control.

Visfatin upregulates expression of MMP2, MMP9, and VEGF mRNA

Invasion and angiogenesis are hallmarks of tumor progression. Tumor invasion is often associated with increased synthesis of matrix metalloproteinases (MMPs), among which MMP2 and MMP9 are of central importance. Vascular endothelial growth factor (VEGF) is one of the most important growth factors for tumor angiogenesis. To investigate whether visfatin regulates the expression of MMP2, MMP9, and VEGF in breast cancer cells, we performed real-time PCR using specific primer sets for these genes. We found that visfatin significantly increased expression of MMP2, MMP9, and VEGF mRNA in MCF-7 cells (Fig. 4), suggesting that it may be a regulator for invasion and angiogenesis of breast cancer.

DISCUSSION

Obesity, with an increased secretion of adipokines (Calle and Thun, 2004), is a worldwide problem that impacts risk and prognosis of several common type cancers. Specially, risk of breast cancer is highly associated with obesity (Stephenson and Rose, 2003). Previous studies have shown that changes in adipokine level affect cell proliferation, apoptosis, invasion, and angiogenesis in the progression of breast cancer (Percik and Stumvoll, 2009; Vona-Davis and Rose, 2007). Visfatin is a newly found candidate for an obesity-induced adipokine involved in the cancer progression, because serum visfatin level is greatly increased in parallel with increase of fat accumulation and obesity (Catalán et al., 2010; Fukuhara et al., 2005), and because it has been well known to be involved in the proliferation of early B cell lines (as PBEF) (Samal et al., 1994) and in the cellular energy metabolism (as Nampt) (Imai, 2009).

In this study we have identified role of visfatin in the breast cancer cell proliferation and cancer progression. Positive association between cancer and serum visfatin level has recently been reported. In gastric cancer patients, the mean serum vis-

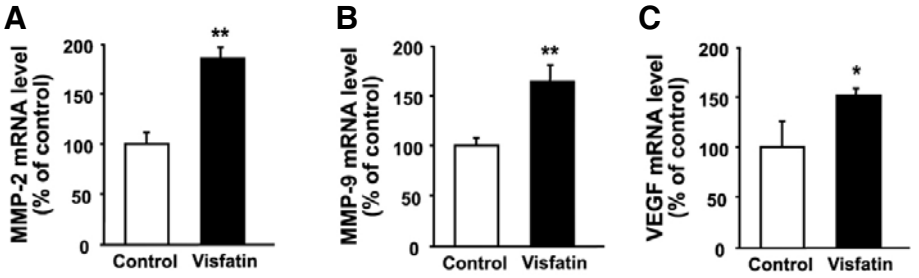

Fig. 4. Effect of visfatin on MMP2, MMP9 and VEGF gene expression. MCF-7 cells were plated in 6-well plates and incubated for 24 h in the presence of visfatin (500 ng/ml) to examine MMP2, MMP9 and VEGF gene expression patterns. Total RNA was extracted from cells and analyzed by real-time PCR. Expression of MMP2 (A), MMP9 (B), and VEGF (C) mRNA species were significantly increased by treatment of MCF-7 cells with visfatin. The data were expressed as mean ± S.E.M (n = 6). *, $p < 0.05$; **, $p < 0.01$ for visfatin vs. control.

fatin level is significantly higher than that of age- and sex-matched controls (Nakajima et al., 2009). Moreover, endogenous visfatin is increased in colorectal tumor tissue compared to normal colorectal mucosa (Hufton et al., 1999). Histochemical analyses have shown that visfatin is highly expressed in the malignant epithelium of human breast neoplasias and prostate adenocarcinomas (Bae et al., 2006; Patel et al., 2010). These earlier reports suggest that visfatin may also be involved in proliferation and progression of breast cancer. To verify this possibility of visfatin action in the breast cancer progression, we first examined change of cell proliferation after treatment of visfatin in MCF-7 cells. In accordant with aforementioned studies showing positive correlation between visfatin and certain cancer cell proliferation, we observed increased proliferation of MCF-7 cells after exposure to visfatin. This increase may be, at least in part, due to an increase in DNA synthesis, because visfatin significantly increased the BrdU incorporation in the cultured MCF-7 cells. The effect of visfatin on DNA synthesis was reconfirmed by an increased proportion of S phase cells treated with visfatin. Previous report also showed that visfatin induced increase in DNA synthesis and proportion of S phase in the cell cycle of rat cardiac fibroblasts (Yu et al., 2010). However, it has not yet been clear how visfatin exerts stimulatory effect on DNA synthesis.

Visfatin increased mRNA levels of cyclin D1 and cdk2, well-known regulators for the G1-S progression (Sherr, 1994), suggesting that stimulatory effect of visfatin on the MCF-7 cell proliferation may be caused by its action on the G1-S phase transition. Recent studies have shown that some other adipokines such as leptin and adiponectin are involved in the G1-S progression of breast cancer (Vona-Davis and Rose, 2007), by regulating expression of cyclin D1 (Dieudonne et al., 2006; Okumura et al., 2002). Further investigation is obviously required to clarify detailed mechanism of visfatin action for the expression of cyclin D1 and cdk2.

This study showed that expression of MMP2 and MMP9 mRNA was increased by treatment of MCF-7 cells with recombinant visfatin, suggesting that visfatin plays a role in the regulation of metastasis in breast cancer cells. Recent studies have shown that visfatin stimulates proliferation of prostate cancer cells as well as synthesis of MMP genes linked to advanced stage cancer, increased tumor cell invasion, and building of metastatic formations (Duffy et al., 2000; Kim et al., 2001; Patel et al., 2010). Moreover, VEGF, a potent pro-angiogenic protein involved in the modulation of tumor growth and progression (Martiny-Baron and Marmé, 1995), was significantly increased in the MCF-7 cells by visfatin treatment. These findings together suggest that visfatin may be involved in the process of metastasis and tumor-related angiogenesis, though we have not performed *in vivo* study to verify this notion. It was recently reported that visfatin stimulates synthesis of pro-angiogenic molecules, such as VEGF and MMPs via mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathways in human endothelial cells (Adya et al., 2008). Therefore, visfatin might elicit changes in gene expression of MMP-2, MMP-9 and VEGF through a similar signaling pathway in the breast cancer cells. Further studies are required for a detailed mechanism of this visfatin action.

Estrogen, a strong mitogen for breast cancer, stimulates proliferation of breast cancer cells (LaMarca and Rosen, 2007; Lee and Nam, 2008). Moreover, estrogen receptor (ER) α regulates expression of genes involved in growth and development of breast cancer, and plays an important role in breast cancer progression (Santen et al., 2009). However, visfatin did not alter expression of ERα mRNA (Supplementary Fig. 1A), suggesting that visfatin stimulates proliferation of MCF-7 cells through a different pathway from estrogen action.

Previous study revealed that visfatin exerted insulin-mimetic effects through binding and activating the insulin receptor (Fukuhara et al., 2005). However, this original paper has been retracted because of concerns on reproducibility of results (Körner et al., 2007; Revollo et al., 2007). Although it remains controversial, our data showed that visfatin does not affect expression of Foxo1, a transcription factor mediating insulin signaling (Kido et al., 2001), in the MCF-7 cells (Supplementary Fig. 1B).

In conclusion, this is the first study to demonstrate that visfatin stimulates MCF-7 cell proliferation. Visfatin may contribute to breast cancer development by increasing the cell proliferation rate through stimulation of cell cycle progression, and by increasing the expression of genes that play roles in metastasis and angiogenesis. Our findings provide further insight into the relation between human breast cancer and obesity.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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REFERENCES

Adya, R., Tan, B.K., Punn, A., Chen, J., and Randeva, H.S. (2008). Visfatin induces human endothelial VEGF and MMP-2/9 production via MAPK and PI3K/Akt signalling pathways: novel insights into visfatin-induced angiogenesis. Cardiovasc. Res. *78*, 356-365.

Bae, S.K., Kim, S.R., Kim, J.G., Kim, J.Y., Koo, T.H., Jang, H.O.,

Yun, I., Yoo, M.A., and Bae, M.K. (2006). Hypoxic induction of human visfatin gene is directly mediated by hypoxia-inducible factor-1. FEBS Lett. *580*, 4105-4113.
Calle, E.E., and Thun, M.J. (2004). Obesity and cancer. Oncogene *23*, 6365-6378.
Catalán, V., Gomez-Ambrosi, J., Rodriguez, A., Ramirez, B., Silva, C., Rotellar, F., Cienfuegos, J.A., Salvador, J., and Fruhbeck, G. (2010). Association of increased Visfatin/PBEF/NAMPT circulating concentrations and gene expression levels in peripheral blood cells with lipid metabolism and fatty liver in human morbid obesity. Nutr. Metab. Cardiovasc. Dis. 25.
Dieudonne, M.N., Bussiere, M., Dos Santos, E., Leneveu, M.C., Giudicelli, Y., and Pecquery, R. (2006). Adiponectin mediates antiproliferative and apoptotic responses in human MCF7 breast cancer cells. Biochem. Biophys. Res. Commun. *345*, 271-9.
Duffy, M.J., Maguire, T.M., Hill, A., McDermott, E., and O'Higgins, N. (2000). Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. Breast Cancer Res. *2*, 252-257.
Fukuhara, A., Matsuda, M., Nishizawa, M., Segawa, K., Tanaka, M., Kishimoto, K., Matsuki, Y., Murakami, M., Ichisaka, T., Murakami, H., et al. (2005). Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science *307*, 426-430.
Garten, A., Petzold, S., Korner, A., Imai, S., and Kiess, W. (2009). Nampt: linking NAD biology, metabolism and cancer. Trends Endocrinol. Metab. *20*, 130-138.
Hufton, S.E., Moerkerk, P.T., Brandwijk, R., de Bruine, A.P., Arends, J.W., and Hoogenboom, H.R. (1999). A profile of differentially expressed genes in primary colorectal cancer using suppression subtractive hybridization. FEBS Lett. *463*, 77-82.
Imai, S. (2009). Nicotinamide phosphoribosyltransferase (Nampt): a link between NAD biology, metabolism, and diseases. Curr. Pharm. Des. *15*, 20-8.
Kang, J.H., Yu, B.Y., and Youn, D.S. (2007). Relationship of serum adiponectin and resistin levels with breast cancer risk. J. Korean Med. Sci. *22*, 117-121.
Karastergiou, K., and Mohamed-Ali, V. (2010). The autocrine and paracrine roles of adipokines. Mol. Cell Endocrinol. *318*, 69-78.
Kido, Y., Nakae, J., and Accili, D. (2001). Clinical review 125: The insulin receptor and its cellular targets. J. Clin. Endocrinol. Metab. *86*, 972-9.
Kim, J.H., Kim, T.H., Jang, J.W., Jang, Y.J., and Lee, S.T. (2001). Analysis of matrix metalloproteinase mRNAs expressed in hepatocellular carcinoma cell lines. Mol. Cells *12*, 32-40.
Körner, A., Garten, A., Bluher, M., Tauscher, R., Kratzsch, J., and Kiess, W. (2007). Molecular characteristics of serum visfatin and differential detection by immunoassays. J. Clin. Endocrinol. Metab. *92*, 4783-4791.
LaMarca, H.L., and Rosen, J.M. (2007). Estrogen regulation of mammary gland development and breast cancer: amphiregulin takes center stage. Breast Cancer Res. *9*, 304.
Lee, S.H., and Nam, H.S. (2008). TNF alpha-induced down-regulation of estrogen receptor alpha in MCF-7 breast cancer cells. Mol. Cells *26*, 285-290.
Loi, S., Milne, R.L., Friedlander, M.L., McCredie, M.R., Giles, G.G., Hopper, J.L., and Phillips, K.A. (2005). Obesity and outcomes in premenopausal and postmenopausal breast cancer. Cancer Epidemiol. Biomarkers Prev. *14*, 1686-1691.
Martiny-Baron, G., and Marme, D. (1995). VEGF-mediated tumour angiogenesis: a new target for cancer therapy. Curr. Opin. Biotechnol. *6*, 675-680.
Nakajima, T.E., Yamada, Y., Hamano, T., Furuta, K., Gotoda, T., Katai, H., Kato, K., Hamaguchi, T., and Shimada, Y. (2009). Adipocytokine levels in gastric cancer patients: resistin and visfatin as biomarkers of gastric cancer. J. Gastroenterol. *44*, 685-690.
Okumura, M., Yamamoto, M., Sakuma, H., Kojima, T., Maruyama, T., Jamali, M., Cooper, D.R., and Yasuda, K. (2002). Leptin and high glucose stimulate cell proliferation in MCF-7 human breast cancer cells: reciprocal involvement of PKC-alpha and PPAR expression. Biochim. Biophys. Acta *1592*, 107-116.
Patel, S.T., Mistry, T., Brown, J.E., Digby, J.E., Adya, R., Desai, K.M., and Randeva, H.S. (2010). A novel role for the adipokine visfatin/pre-B cell colony-enhancing factor 1 in prostate carcinogenesis. Peptides *31*, 51-57.
Percik, R., and Stumvoll, M. (2009). Obesity and cancer. Exp. Clin. Endocrinol. Diabetes *117*, 563-566.
Porter, G.A., Inglis, K.M., Wood, L.A., and Veugelers, P.J. (2006). Effect of obesity on presentation of breast cancer. Ann. Surg. Oncol. *13*, 327-332.
Revollo, J.R., Korner, A., Mills, K.F., Satoh, A., Wang, T., Garten, A., Dasgupta, B., Sasaki, Y., Wolberger, C., Townsend, R.R., et al. (2007). Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab. *6*, 363-375.
Samal, B., Sun, Y., Stearns, G., Xie, C., Suggs, S., and McNiece, I. (1994). Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol. Cell. Biol. *14*, 1431-1437.
Santen, R., Cavalieri, E., Rogan, E., Russo, J., Guttenplan, J., Ingle, J., and Yue, W. (2009). Estrogen mediation of breast tumor formation involves estrogen receptor-dependent, as well as independent, genotoxic effects. Ann. NY Acad. Sci. *1155*, 132-140.
Sherr, C.J. (1994). G1 phase progression: cycling on cue. Cell *79*, 551-555.
Stephenson, G.D., and Rose, D.P. (2003). Breast cancer and obesity: an update. Nutr. Cancer *45*, 1-16.
Vona-Davis, L., and Rose, D.P. (2007). Adipokines as endocrine, paracrine, and autocrine factors in breast cancer risk and progression. Endocr. Relat. Cancer *14*, 189-206.
Yu, X.Y., Qiao, S.B., Guan, H.S., Liu, S.W., and Meng, X.M (2010). Effects of visfatin on proliferation and collagen synthesis in rat cardiac fibroblasts. Horm. Metab. Res. *42*, 507-13.