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Evaluation of Human Breast Adenocarcinoma (MCF-7) Cells Proliferation in Co-Culture with Human Adipocytes in Three Dimensional Collagen Gel Matrix: Norepinephrine as a Lipolytic Factor

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ABSTRACT

Background: Norepinephrine plays a trophic role in the control of cell replication and differentiation in target cells that express adrenergic receptors. **Methods:** In this study, we have tested the influence of infraphysiological, physiological and supraphysiological concentrations (0.0001 nM, 1 nM, 10000 nM) of human norepinephrine on the proliferation of breast cancer cells (human breast adenocarcinoma [MCF-7]) in co-culture with human adipocytes in three-dimensional collagen gel matrix culture. Cell proliferation and lipolysis rate were measured by 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) and Oil red O colorimetric assay in second, 7th and 14th days of culture experiments. **Results:** Our results showed a direct correlation between lipolysis rate of adipocytes and proliferation rate of MCF-7 cells. Both physiological and supraphysiological concentrations of human norepinephrine significantly ($P<0.05$) increased the proliferation of MCF-7 cells synchronously with progress of adipocyte lipolysis. The proliferations of MCF-7 cells were significantly decreased after conversion of adipocytes to fibroblast-like cells by supraphysiological concentration of norepinephrine. There was no statistical difference in lipolysis of adipocytes and proliferation of MCF-7 cells in response to infraphysiological concentration of norepinephrine. **Conclusion:** These findings indicated that norepinephrine stimulated the proliferation of MCF-7 cells in co-culture with human adipocytes as a lipolytic factor and that norepinephrine effect was suppressed by conversion of adipocytes to fibroblast-like cells, suggesting adipocytes as another target for prevention and therapy of breast cancer. *Iran. Biomed. J. 10 (3): 125-131, 2006*

Keywords: Human breast adenocarcinoma (MCF-7) cell, Human adipocyte, Norepinephrine, Proliferation, Lipolysis

INTRODUCTION

Breast cancer is the most common malignancy in women and comprises 18% of all cancers [1]. The past few years have provided substantial evidence for the vital role of the local environment of an emerging tumor for various steps of tumor genesis, including proliferation and local invasion. Phenotypic behavior of malignant cells is regulated not only by cell autonomous signals, but also by effects exerted from surrounding stromal cells [2]. In breast tissue, the main stromal cell types are fibroblasts and adipocytes. Fibroblasts are widely accepted as promoters of the invasive growth of breast carcinoma cells [3]. In the adipose tissue of the breast, adipocytes and preadipocytes exist as mature and immature cells, respectively. The former constitutes most of the adipose tissue and has a large lipid droplet in the cytoplasm and the latter is fibroblast-like and has minute lipid droplets [4]. Emerging, although controversial, evidence suggests that exposure to various forms of stressors may

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influence vulnerability to breast cancer and affect length of survival once a particular tumor has developed [5]. Norepinephrine is a catecholamine released into the blood stream in response to stressful situations. Norepinephrine can cause many reactions in the body; however, one of its main functions is to increase fat metabolism in adipose tissue [6]. In addition to its role as a neurotransmitter and 'stress' hormone, norepinephrine plays a trophic role in the control of cell replication and differentiation in target cells that express adrenergic receptors [7].

In mammals, 'spikes' of adrenergic activity also modulate the rate of cell replication and differentiation and thus control the architectural modeling of adrenergic target tissues [8]. Many cell types, including adipocytes [9] and epithelial cancers, express ß-adrenergic receptors [10] which can once again resume their role in the control of cell replication and differentiation [11]. Adipocytes have some hormone receptors which are metabolically active. Lipogenesis is accelerated by insulin [8] and lipolysis is stimulated by norepinephrine, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH) or gonadotropic hormone (GH) [12]. During lipolysis, triglycerides in adipocytes are hydrolyzed to glycerol and free fatty acids [13]. The effect of catecholamine on cell proliferation and/or metastatic capacity of tumors may be direct, acting on tumoral cells, or indirect, mediated by other mechanisms including the immunological system [14].

Recently, some studies have demonstrated that adipocytes promoted the growth of breast carcinoma cells but preadipocytes had the opposite effect, inhibiting proliferation of breast carcinoma cells [15-17]. The *in vivo* studies revealed that norepinephrine treatment resulted in a significant suppression of breast tumor growth [18-20], but *in vitro* studies showed that norepinephrine stimulated breast cancer cells proliferation [7, 18, 21]. Based on all the considerations outlined above**,** this study was designed to obtain experimental evidence to determine whether norepinephrine as a lipolytic factor had an indirect role in proliferation of breast carcinoma cells (human breast adenocarcinoma [MCF-7]) in co-culture with human adipocytes. For this purpose, we used an *in vitro* three dimensional collagen gel matrix system, a close resemblance to *in vivo* environment.

MATERIALS AND METHODS

Cell line and preparation of adipocytes. As typical breast carcinoma cells, we used MCF-7 (NCBI C135) breast cancer cell line (obtained from National Cell Bank of Iran, Tehran). Human adipose tissue was obtained from the abdominal subcutaneous region (epigastric region of the abdominal wall) from elective or laparoscopic abdominal surgery (hernia, gall stone, etc.) performed at Imam Khomeini Hospital (Tehran, Iran). Except for obesity and minor metabolic disturbances, the subjects were healthy and took no regular medication. Informed consent was obtained from the subjects before the surgical procedure. After being removed, adipose tissue samples of 5 to 20 g were immediately transferred under sterile conditions to the laboratory.

The subcutaneous adipose tissue specimens were dissected from fibrous material and visible blood vessels, minced into small pieces, and digested in phosphate-buffered saline (0.15 M, pH 7.2) containing 20 mg/ml BSA and 250U/mL collagenase type I (Sigma, USA) at 37°C for 90 minutes. The completely disaggregated tissue was centrifuged at 200 × *g* for 10 minutes. The top layer of mature adipocytes was collected in a thin, white, floating layer and filtered through 200 µm and 150 µm nylon meshes to minimize contaminating preadipocytes or fibroblasts. Adipocyte cell were stained with Oil red O (Sigma, USA) and counted using a hemacytometer [4].

Adipocytes growth arrest. Adipocytes used in co-culture were growth arrested by treatment with 20 µg/ml mitomycin C (Roche Diagnostic, Germany) for 2 h and then washed three times by DMEM–Ham's F-12 medium (vol/vol, 1:1) (Sigma, USA) [22, 23].

Three-dimensional collagen gel culture system. MCF-7 breast cancer cells (1×10^4) with 1×10^5 mitomycin C treated mature adipocytes were co-cultured in three-dimensional collagen gel matrix. The results were obtained from cultured material after 2, 7 and 14 days. The collagen culture system was prepared as follows: Briefly, 8 volumes of type I collagen (Sigma, USA) were mixed with 1 volume of ten-fold concentrated Ham's F-12 medium and 1 volume of a reconstruction buffer (2.2 g of $NaHCO_3$ and 4.77 g of HEPES in 100 ml of 0.05 N NaOH). This collagen gel solution (90 µl) was mixed with the 1×10^5 mitomycin C treated adipocytes and

1×10^4 MCF-7 breast cancer cells. This collagen gel solution containing cells was poured into each well of a 24-well cell culture plate (Sigma, USA). The culture plate was immediately warmed to 37°C to allow a gel to form. The gel was further covered with 400 µl per well Ham's F-12 medium supplemented with 15% newborn calf serum (Sigma, USA) and 50 µg/ml gentamicin (Sigma, USA) for 24 h. The gel was washed three times the next day with PBS and then maintained in media in the absence (control) or presence of different concentration of human norepinephrine (0.0001 nM, 1 nM and, 10,000 nM) (Sigma, USA). Medium was changed every two days [15]. The experiment was repeated three times.

Cell proliferation assay. Cell proliferation was measured by 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) colorimetric assay after 2, 7 and 14 days of culture experiments. Briefly, 50 mg of MTT (Sigma, USA) was dissolved in 10 ml PBS buffer (0.15 M, pH 7.2) to prepare MTT stock solution (5 mg/ml); 500 µl of this solution was added to each well of a 24-well cell culture plate with collagen gel containing cells (adipocytes and MCF-7) and plates were incubated for 7 h at 37°C. Supernatant was removed and 500 µl of 0.04 N HCl in isopropanol was added to each well of a 24-well cell culture plate for 30 minutes. Then, samples were transferred from each plate into a 96-microtiter plate (Sigma, USA) before reading optical density at 580 nm with an ELISA plate reader (Labsystems Multiscan RC). A standard curve was prepared utilizing a known concentration of cells before each experiment. The MTT test assesses cell metabolism based on the ability of the mitochondrial succinate-tetrazolium reductase system to convert the yellow compound MTT to a blue formazan dye. The amount of dye produced is proportional to the number of live metabolically active cells. The proliferation proportion (%P) of MCF-7 breast cancer cell in co-culture was calculated according to the following formula [24]:

$$\%P_{\text{Experiment}} = [OD_{\text{experiment}} - OD_{\text{control}} / OD_{\text{control}}] \times 100$$

Oil red O staining and measurement. Lipogenesis and lipolysis were evaluated by staining accumulated neutral lipids with Oil red O [25]. Collagen gels contained cultured cells were fixed in 4% formaldehyde, washed in water and stained with a 0.6% (w/v) oil red O solution (60% isopropanol, 40% water) for 1 h at room temperature. For quantification, cells were washed extensively with water to remove unbound dye; then 1 ml of isopropyl alcohol was added to the stained culture dish. After 1 h, the absorbance of the extract was assayed by a spectrophotometer at 510 nm. The lipolysis rate was calculated according to the following formula [26]:

$$\text{Lipolysis} = [OD_{\text{control}} \_ OD_{\text{Experiment}}]/OD_{\text{Experiment}} \times 100$$

Histologic examination. Collagen gels that contained cultured cells were fixed with 10% formalin solution, embedded in paraffin wax, and routinely processed. The deparaffinized sections were stained with Oil Red O and Mayer's hematoxylin [15]. The images were obtained using a light microscope (Olympus 1 × 70, Japan) and the Magnifier imaging system.

Statistical analysis. Statistical comparisons were done using one-way analysis of variance (ANOVA), followed by the Tukey- Kramer test for multiple comparisons among the groups.

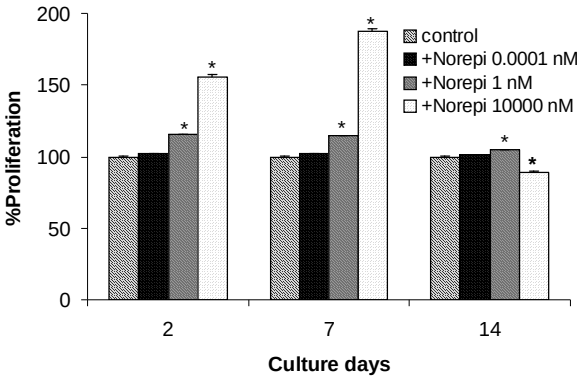

Fig. 1. Growth responses of MCF-7 cells were plated (1×10^4/well) in co-culture with mitomycin C treated adipocytes (1×10^5/well) in collagen gel matrix culture and treated with verified concentrations of norepinephrine. The MTT assay was performed at 2nd, 7th and 14th day of culture. Results are expressed as the percentages of cell numbers. Untreated MCF-7 cells were used as a control. Data are expressed as mean ± SEM of triplicate incubation. *Significant difference at $P<0.05$.

RESULTS

Cell Proliferation. To study the effect of norepinephrine on human breast carcinoma, we evaluated the proliferation of MCF-7 cell line in co-culture with mitomycin C treated adipocytes incubated in the presence of different concentration

of norepinephrine by colorimetric MTT assay. As can be seen in Figure 1, there was no statistical difference in MCF-7 cell proliferation in response to infraphysiological concentration of norepinephrine (0.0001 nM). The physiological concentration of norepinephrine (1 nM) caused a significant (P<0.05) increase in proliferation of MCF-7 cells to about 15.7% and 15% more than control cells at the 2nd and 7th day after cell culture. There was no significant difference in MCF-7 cell proliferation at the 14th day of culture (Fig. 1). The supraphysiological concentration of norepinephrine (10,000 nM) caused a significant (P<0.05) increase in proliferation of MCF-7 cells to about 55.5% and 87.5% more than control cells at the 2nd and 7th day after culture, respectively; but at the 14th day of culture it caused a significant (P<0.05) decrease in proliferation of MCF-7 cells to about 11.7% less than control cells (Fig. 1).

Effects of norepinephrine on lipolysis of the human adipocyte. Oil red O staining and measurement at 2, 7 and 14 days after cell culture showed that the effect of norepinephrine on lipolysis of adipocytes depended on concentration. There was no statistical difference in lipolysis of adipocytes in response to infraphysiological concentration of norepinephrine (0.0001 nM). The physiological concentration of norepinephrine (1 nM) caused a significant (P<0.05) increase in lipolysis of adipocytes to about 7.75%, 7.5% and 10.75% more than control cells at the second, 7th and 14th day of culture, respectively. The supraphysiological concentration of norepinephrine (10,000 nM) caused a significant (P<0.05) increase in lipolysis of adipocytes to about 39.5%, 96.25% and 98% more than control cells in the second, 7th and 14th days of culture, respectively (Fig. 2).

Morphology of MCF-7 cells. MCF-7 cells co-cultured with adipocytes adhered to mature adipocytes and organized around spherical adipocytes at day 0 (Fig. 3 A and B). When exposed to supraphysiological concentration of norepine-phrine, the single lipid droplet of adipocytes displayed widespread cytoplasm with fine granular lipid droplets at day two (Fig. 3C). At day seven, the morphology of human adipocytes changed to fibroblast-like cells (Fig. 3D). As time progressed, fibroblast-like cells maintained their morphology and MCF-7 cells separated from them completely (Fig. 3E). When exposed to physiological concentration of norepinephrine, the a single lipid droplet of adipocytes displayed widespread cytoplasm with fine granular lipid droplets at day seven; no other events were observed in the remaining culture time (Fig. 3C). We did not observe any morphologic changes in adipocytes and MCF-7 cells by administration of infraphysiological concentration of norepinephrine.

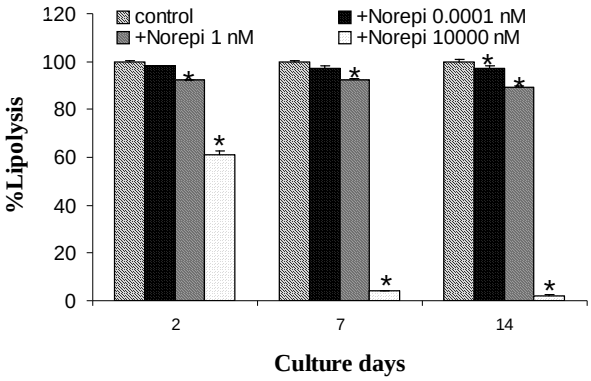

Fig. 2. Effects of different concentrations of norepinephrine on adipocyte lipolysis plated (1×10^5/well) in co-culture with MCF-7 cells (1×10^4/well) in collagen gel matrix. Oil red O measurement was performed at 2nd, 7th and 14th day of culture. Results are expressed as the percentages of absorbance of Oil red O staining that exhibited amount of lipid content in each group. The untreated group was used as a control. Data are expressed as mean ± SEM of triplicate incubation. *Significant difference at P<0.05.

DISCUSSION

In this study, we showed that norepinephrine stimulated the proliferation of MCF-7 cells in co-culture with adipocytes as a lipolytic factor and inhibited the proliferation of cells when adipocytes lost their lipid contents and were converted to fibroblast-like cells. There was also a direct correlation between lipolysis rate of adipocytes and proliferation rate of MCF-7 cells. The growth of primary and metastatic carcinomas is not simply the result of autonomous growth of malignant epithelial cells, but it requires the interaction between the various cell types within the tumor, including stromal cells [27]. In the breast, the main stromal cell types are fibroblasts and adipocytes and preadipocytes [3]. Breast tumors are characterized by the accumulation of fibroblasts adjacent to malignant epithelial cells, which is commonly known as the desmoplastic reaction. In fact, the majority of breast cancers have been referred to as "scirrhous" because of their extremely hard

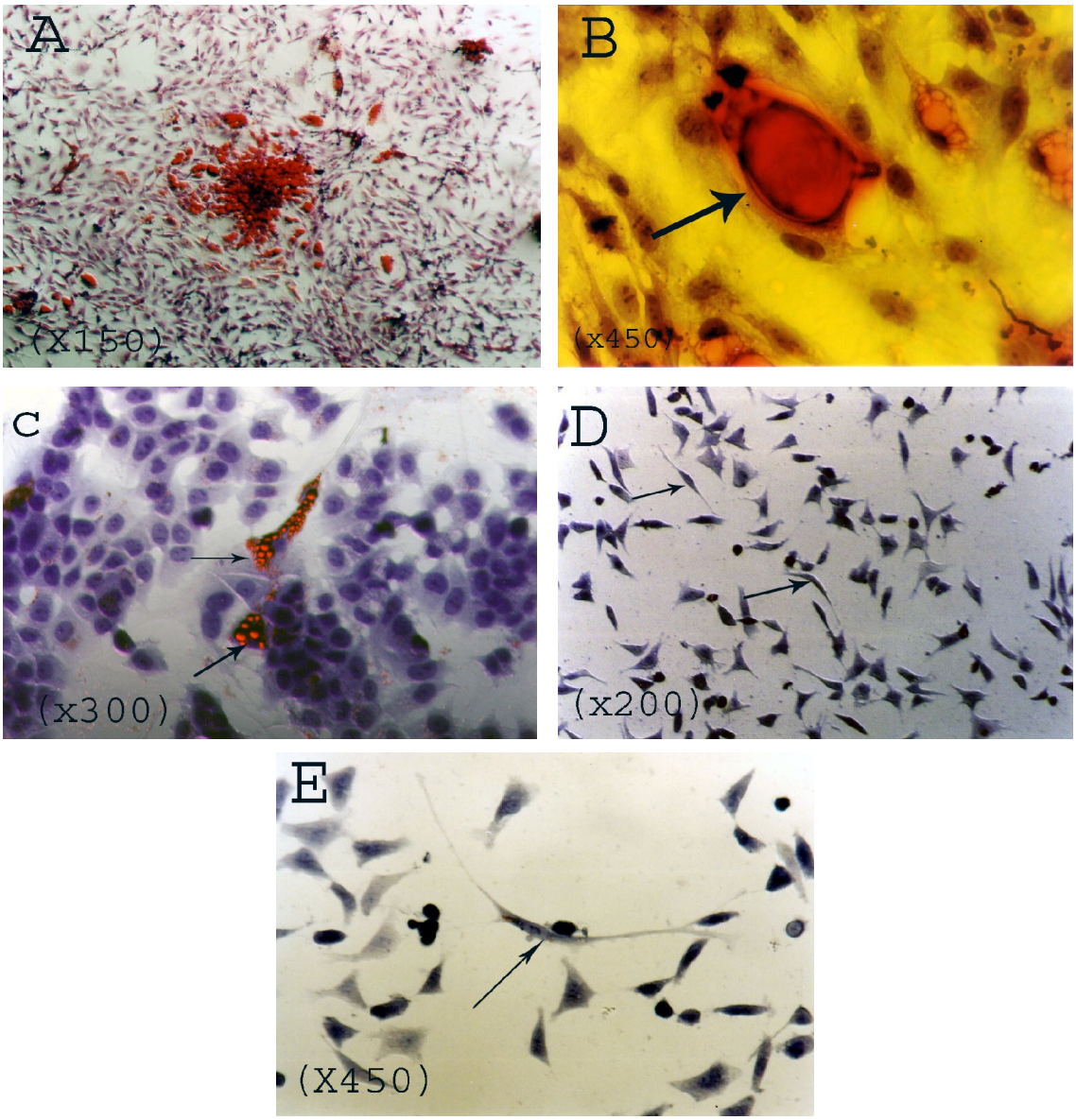

Fig. 3. MCF-7 cells in co-culture with human adipocytes, treated with verified concentrations of norepinephrine and stained by Oil red O and Hematoxylin. **(A)** and **(B)**, Human mature adipocytes (arrow) in co-culture with MCF-7 cells at day 0. Mature adipocytes were spherical with large lipid droplet. MCF-7 cells adhered to mature adipocytes and organized on the surface of adipocytes. **(C)**, Human mature adipocytes underwent lipolysis when treated with norepinephrine. Unilocular human mature adipocytes converted to multilocular adipocytes (arrow) in this condition. **(D)** and **(E)**, Mature human adipocytes converted into fibroblast-like cells (arrow) after treatment with supraphysiological concentration of norepinephrine. MCF-7 cells did not adhere to fibroblast-like cells.

consistency provided by large numbers of fibroblasts dispersed between malignant epithelial cells, as well as within the immediate periphery of tumors. Evidence from several laboratories indicates that these are fibroblast-like cells originate from adipose tissue and most likely represent potential preadipocytes [28].

Previous studies have demonstrated that adipocytes promoted the growth of breast carcinoma cells, but preadipocytes had the opposite effect and inhibited proliferation of breast carcinoma cells [15-17]. Numerous studies have provided evidence that norepinephrine has a stimulatory effect on the proliferation of the breast cancer cells [7, 18, 21, 29]. In contrast, some researchers have reported that norepinephrine has an inhibitory effect on the growth of breast carcinoma [18, 20, 30, 31]. When the current and previous results are put together, it is

apparent that our results support the hypothesis that during carcinogenesis, the cancer epithelial cells invade the surrounding stromal tissue while the number of mature adipocytes progressively decreases and fibroblast-like cells progressively increase [32].

The mechanism by which norepinephrine promotes the growth of MCf-7 breast cancer cell line in co-culture with adipocytes is unclear from our study. It has recently been shown that norepinephrine induces lipolysis through three subtypes of ß-adrenoceptors expressed in the adipocytes. During lipolysis, triglycerides in adipocytes are hydrolyzed to glycerol and free fatty acids [13]. Increased fatty acids are used to meet the increased energy demands imposed by the breast cancer cells [34].

Another interesting finding of this study is the observation that exposure of adipocytes to norepinephrine in co-culture with MCF-7 cells increases the degradation of fatty acids, reducing their availability to synthesize triglycerides. These results are in agreement with findings from the recent literature. Human adipocytes may play a role in the metabolic alterations observed during stress reactions and in certain forms of cachexia [34, 35]. Cachexia is a progressive wasting syndrome characterized by extensive loss of adipose tissue. It occurs in about half of all cancer patients. One candidate for this effect is a tumor lipid mobilizing factor, which stimulates lipolysis directly through a cyclic AMP-mediated process via interaction with a beta3-adrenergic receptor [35].

These findings indicate that norepinephrine stimulated the proliferation of MCF-7 cells in co-culture with human adipocytes as a lipolytic factor, and that norepinephrine effect was suppressed by conversion of adipocytes to fibroblast-like cells. This suggests that prevention and therapy of breast cancer may be targeted not only to cancer cells, but also to adipocytes themselves.

REFERENCES

1. McPherson, K., Steel, C.M. and Dixon, J.M. (2000) ABC of the breast diseases, breast cancer epidemiology, risk factors and genetics. *BMJ 321(7261): 624-628*.
2. Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell 100 (1): 57-70*.
3. Ruohola, J.K., Viitanen, T.P., Valve, E.M., Seppanen, J.A., Loponen, N.T., Keskitalo, J.J., Lakkakorpi, P.T. and Harkonen, P.L. (2001) Enhanced invasion and tumor growth of fibroblast growth factor 8b-overexpressing MCF-7 human breast cancer cells. *Cancer Res. 61 (10): 4229-4237*.
4. Tholpady, S.S., Aojanepong, C., Llull, R., Jeong, J.H., Mason, A.C., Futrell, J.W., Ogle, R.C. and Katz, A.J. (2005) The cellular plasticity of human adipocytes. *Ann. Plast. Surg. 54 (6): 651-656*.
5. Hilakivi-Clarke, L., Rowland, J., Clarke, R. and Lippman, M.E. (1994) Psychosocial factors in the development and progression of breast cancer. *Breast Cancer Res. Treat. 29 (2): 141-160*.
6. McCarty, M.F. (2001) Modulation of adipocyte lipoprotein lipase expression as a strategy for preventing or treating visceral obesity. *Med. Hypotheses 57 (2): 192-200*.
7. Slotkin, T.A., Zhang, J., Dancel, R., Garcia, S.J., Willis, C. and Seidler, F.J. (2000) Beta-adrenoceptor signaling and its control of cell replication in MDA-MB-231 human breast cancer cells. *Breast Cancer Res. Treat. 60 (2): 153-166*.
8. Slotkin, T.A., Levant, B., Orband-Miller, L., Queen, K.L. and Stasheff, S. (1988) Do sympathetic neurons coordinate cellular development in the heart and kidney? Effects of neonatal central and peripheral catecholaminergic lesions on cardiac and renal nucleic acids and proteins. *J. Pharmacol. Exp. Ther. 244 (1): 166-172*.
9. Tavernier, G., Jimenez, M., Giacobino, J.P., Hulo, N., Lafontan, M., Muzzin, P. and Langin, D. (2005) Norepinephrine induces lipolysis in beta1/beta2/ beta3-adrenoceptor knockout mice. *Mol. Pharmacol. 68 (3): 793-799*.
10. Mitra, S.P. and Carraway, R.E. (1999) Synergistic effects of neurotensin and b-adrenergic agonist on 30, 50-cyclic adenosine monophosphate accumulation and DNA synthesis in prostate cancer PC3 cells. *Biochem. Pharmacol. 57 (12): 1391-1397*.
11. Re, G., Badino, P., Novelli, A., Girardi, C. and DiCarlo, F. (1996) Evidence for functional ß-adrenoceptor subtypes in CG-5 breast cancer cells. *Pharmacol. Res. 33(4-5): 255-260*.
12. Sugihara, H., Yonemitsu, N., Miyabara, S. and Toda, S. (1987) Proliferation of unilocular fat cells in the primary culture. *J. Lipid Res. 28 (9): 1038-1045*.
13. Wahrenberg, H., Lonnqvist, F. and Arner, P. (1989) Mechanisms underlying regional differences in lipolysis in human adipose tissue. *J. Clin. Invest. 84 (2): 458-467*.
14. Vendewalle, B., Revillion, F. and Lefebvre, J. (1990) Functional ß- adrenergic receptors in breast cancer cells. *J. Cancer Res. Clin. Oncol. 116 (3): 303-306*.
15. Manabe, Y., Toda, S., Miyazaki, K. and Sugihara, H. (2003) Mature adipocytes, but not preadipocytes, promote the growth of breast carcinoma cells in collagen gel matrix culture through cancer-stromal cell interactions. *J. Pathol. 201 (2): 221-228*.
16. Iyengar, P., Combs, T.P., Shah, S.J., Gouon-Evans, V., Pollard, J.W., Albanese, C., Flanagan, L.,

Tenniswood, M.P., Guha, C., Lisanti, M.P., Pestell, R.G. and Scherer, P.E. (2003) Adipocyte-secreted factors synergistically promote mammary tumorigenesis through induction of anti-apoptotic transcriptional programs and proto-oncogene stabilization. *Oncogene 22 (41): 6408-6423*.

17. Rahimi, N., Tremblay, E., McAdam, L., Roberts, A. and Elliott, B. (1998) Autocrine secretion of TGF-beta 1 and TGF-beta 2 by pre-adipocytes and adipocytes: a potent negative regulator of adipocyte differentiation and proliferation of mammary carcinoma cells. *In vitro Cell Dev. Biol. Anim. 34 (5): 412-420*.
18. Vazquez, S.M., Pignataro, O. and Luthy, I.A. (1999) Alpha2-adrenergic effect on human breast cancer MCF-7 cells. *Breast Cancer Res. Treat. 55 (1): 41-49*.
19. Cooper, C.L., Faragher, E.B. (1993) Psychosocial stress and breast cancer: the inter-relationship between stress events, coping strategies and personality. *Psychol. Med. 23 (3): 653-662*.
20. Lang, K., Drell, T.L., Lindecke, A., Niggemann, B., Kaltschmidt, C., Zaenker K.S and Entschladen F. (2004) Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. *Int. J. Cancer 112 (2): 231-238*.
21. Cakir, Y., Plummer, H.K., Tithof, P.K. and Schuller, H.M. (2002) Beta-adrenergic and arachidonic acid-mediated growth regulation of human breast cancer cell lines. *Int. J. Oncol. 21 (1): 153-157*.
22. Heng, B.C., Liu, H. and Cao, T. (2005) Transplanted human embryonic stem cells as biological 'catalysts' for tissue repair and regeneration. *Med. Hypotheses. 64 (6): 1085-1088*.
23. Papini, S., Rosellini, A., Nardi, M., Giannarini, C. and Revoltella, R.P. (2005) Selective growth and expansion of human corneal epithelial basal stem cells in a three-dimensional-organ culture. *Differentiation 73 (2-3): 61-68*.
24. Song, D., Wang, H.L., Wang, S. and Zhang, X.H. (2005) 5-Hydroxytryptamine-induced proliferation of pulmonary artery smooth muscle cells are extracellular signal-regulated kinase pathway dependent. *Acta. Pharmacol. Sin. 26 (5): 563-567*.
25. Ryden, M., Dicker, A., Gotherstorm, C., Tammick, C., Arner, P. and Le Blank, K.(2003) Functional characterization of human mesenchymal stem cell-derived adipocytes. Biochem. *Biophys. Res. Commun. 311 (2): 391-397*.
26. Li, X.H., Zhang, J.C., Sui, S.F. and Yang, M.S. (2005) Effect of daidzin, genistin, and glycitin on osteogenic and adipogenic differentiation of bone marrow stromal cells and adipocytic transdifferentiation of osteoblasts. *Acta. Pharmacol. Sin. 26 (9): 1081-1086*.
27. Van den Hooff, A. (1988) Stromal involvement in malignant growth. *Adv. Cancer Res. 50: 159-96*.
28. Meng, L., Zhou, J., Sasano, H., Suzuki, T., Zeitoun, KM. and Bulun, S.E. (2001) Tumor necrosis factor alpha and interleukin 11 secreted by malignant breast epithelial cells inhibit adipocyte differentiation by selectively down-regulating CCAAT/enhancer binding protein alpha and peroxisome proliferator-activated receptor gamma: mechanism of desmoplastic reaction. *Cancer Res. 61 (5): 2250-2255*.
29. James, G.D., Berge-Landry, H.V.H., Valdimarsdottir, H.B., Montgomery, G.H. and Bovbjerg, D.H. (2004) Urinary catecholamine levels in daily life are elevated in women at familial risk of breast cancer. *Psychoneuroendocrinology 29 (7): 831-838*.
30. Bertin, B., Jockers, R., Strosberg, A.D. and Marullo, S. (1997) Activation of a beta 2-adrenergic receptor/Gs alpha fusion protein elicits a desensitization-resistant cAMP signal capable of inhibiting proliferation of two cancer cell lines. Receptors. *Channels 5 (1): 41-51*.
31. Scriba, D., Aprath-Husmann, I., Blum, W.F. and Hauner, H. (2000) Catecholamines suppress leptin release from *in vitro* differentiated subcutaneous human adipocytes in primary culture via beta1- and beta2-adrenergic receptors. *Eur. J. Endocrinol. 143 (3): 439-445*.
32. Zangani, D., Darcy, K.M., Masso-Welch, P.A., Bellamy, E.S. and Desole, M.S. (1999) Multiple differentiation pathways of rat mammary stromal cells *in vitro*: acquisition of a fibroblast, adipocyte or endothelial phenotype is dependent on hormonal and extracellular matrix stimulation. *Differentiation 64 (2): 91-101*.
33. Tisdale, M.J. (2004) Cancer cachexia. *Langenbecks Arch. Surg. 389 (4): 299-305*.
34. Path, G., Bornstein, S.R., Gurniak, M., Chrousos, G.P., Scherbaum, W.A. and Hauner, H. (2001) Human breast adipocytes express interleukin-6 (IL-6) and its receptor system: increased IL-6 production by beta-adrenergic activation and effects of IL-6 on adipocyte function. *J. Clin. Endocrinol. Metab. 86 (5): 2281-2288*.
35. Tisdale, M.J. (2003) Pathogenesis of cancer cachexia. *J. Support. Oncol. 1 (3): 159-168*.