Cancer cell research 8 (2015) 192-196

www.cancercellresearch.org Article

Influence of IGF - IR on breast cancer cells MDA - MB - 231 biological behavior

Xinxin He, Ximing Shen*, Dongxia Wu, Qing Sun

Department of Pathology, the Sun yat-sen memorial hospital of Sun Yat-Sen University, Guangdong, China, 510120

Abstract: **IGF-IR Antisense Oligodeoxynucleotide (ASODN) was synthesized and added to breast cancer MDA-MB-231 cells to see its influence on cell proliferation and angiogenesis. After the transfection of IGF-IR ASODN on the MDA-MB-231 cells, immunocytochemical method, western blot and RT-PCR methods were used to test the IGF-IR, VEGF and PCNA level. Cell proliferation was analyzed by MTT method. The results showed that after transfection 2-8 μM IGF-IR ASODN can inhibit expression of IGF-IR and cell proliferation. The inhibition effect begun at 24 hours and lasted to 96 hours.The inhibition effect was related to the concentration. IGF-IR ASODN could also inhibit protein level of VEGF and PCNA. Promoting autocrine and paracrine, IGF-IR is important to breast cancer cells growth and angiogenesis ability.**

Keywords: IGF-IR; Breast cancer; Proliferation; Angiogenesis

Received 5 October 2015, Revised 22 November 2015, Accepted 25 November 2015

* Corresponding Author: Ximing Shen, 563389341@qq.com

1. Introduction

Insulin-like growth fac-tors-1(IGF-I) is a kind of cell factor which can effectively promote mitosis and proliferation of cells and angiogenesis [1]. IGF-1 gene is the first confirmed endogenous imprinted genes, its gene lost already in breast cancer was found in a variety of tumor cells. IGF-2 factor, as a kind of multifunctional cell growth regulation of cell proliferation, differentiation, apoptosis, it plays a regulatory role and can also be with other factors in breast cancer mechanism. Malignant tumor incidence and mortality are very high in our country, and are already late for clinic diagnosis [2-4]. Therefore, it needs to strengthen research tumor etiology, pathogenesis, diagnosis and treatment, and improve the early diagnosis and curative effect. In recent years a large number of studies have shown that IGFs levels in the blood had significantly positive correlation with malignant tumor incidence of breast cancer, lung cancer, liver cancer, gastric cancer, colon cancer and other malignant tumor [5-7]. The study tested the influence of IGF-IR ASODN transfected MDA-MB-231 cells in different concentrations on angiogenesis to evaluate effect of IGF-IR on angiogenesis of malignant tumor.

2. Materials and methods

2.1. Agents and materials

The design and synthesis of IGF-IR ASODN are finished by Shanghai Bioengineering and breast cancer MDA-MB-231 cells are kept in our lab. Cell incubator is made by Napco of America. Inverted Microscopic is made by Olympus of Japan.

2.2. Synthesization of IGF-IR ASODN

According to IGF-IRcDNA order, ASODN are synthesized: 5'-T#C#C TCC GGA GCC AGA C#T#T-3' [10]. # represents base with thiophosphoric acid. Bases with thiophosphoric acid can solve the problem that IGF-IR can be dissolved in vivo and strengthen the stability of IGF-IR. This study synthesized IGF-IR ASODN with 5' and 'bases. The "-OH" between the two bases were replaced by "-SH". IGF-IR was synthesized by Shanghai Bioengineering with automated DNA synthesizer.

2.3. Breast cancer MDA-MB-231 cell culture

Breast cancer MDA-MB-231 cell was cultured in complete medium containing 10% CS DMEM and kept in saturation humidity, 37℃ and 5% CO2 incubator. Culture fluid was renewed once in 2days. The cells were digested by 0.25% trypsinase and passaged in the logarithm growth period. All the cells were cultured to logarithm growth period, digested and implanted. The cells were cultured in conditioned medium including 0.1% bovine serum albumin, 1% calf serum, 1.0μmolgreen copperas and IGF-I 10ng/ml after adherent culture so as to reduce influence of serum on cells and complete experiment successfully.

2.4. RT-PCR method

Cells are regulated commonly and added with 1 ml TRIZOL for schizolysis. After being shaken and stewing for 10 minutes, cells were added with 200ml phenol chloroform and being shaken and centrifuged to give convenience to take supernatant materials. We used isopropanol to settle supernatant materials and 75% ethanol to extract Ribonucleic Acid (RNA). Premier was determined according to real-time quantification. Premier of 18sRNAinternal standard: P1: 5c-GTAACCCGTTGAACCCCATT-3c, P2: 5c-CCATC-CAATCGGTAGTAGCG-3c. The premier length is 151bp. Premier of IGF-IR: P1:

5c-AGCTCGGTCAGACAGGATGG-3c, P2: 5c-TTC-GAAGATTAGTTGGTCCAGC-3c. The premier length is 165bp. 1~5Lg total RNA was added into the reaction pipe. The pipe was heat to 75℃ and lasted for 5 minutes and put on ice quickly. Adding 4L 5×buffer and 2L dNTP and heat the pipe to 95℃ and lasted for 5 minutes, and then 94℃ for 30 seconds, and then 60℃ for 30 seconds, and then72℃ for 30 seconds. Heating circulation was 40 times. The collected pure PCR product was taken as relative quantitative criteria product. IGF-IR and 18sr RNA was taken as a positive model and diluted according to 10-5 and 10-9 and relative copy number was determined. Quantitative reaction was finished and standard cure was achieved. The pipe was heat to 95℃ and lasted for 4 minutes, and then 95℃ for 20 seconds, and then 59℃ for 20 seconds. Finished the heating circulation was 40 times. Lastly, the pipe was heated to 94℃ and lasted for 0 second, and then 60℃ for 15 seconds, and then 95℃ for 0 second. Fluorescence signal was collected and analyzed with solubility curve. Every sample included 3 wells. Standard curve and solubility curve were finished and sample relative quantitative copy number was determined by the data.

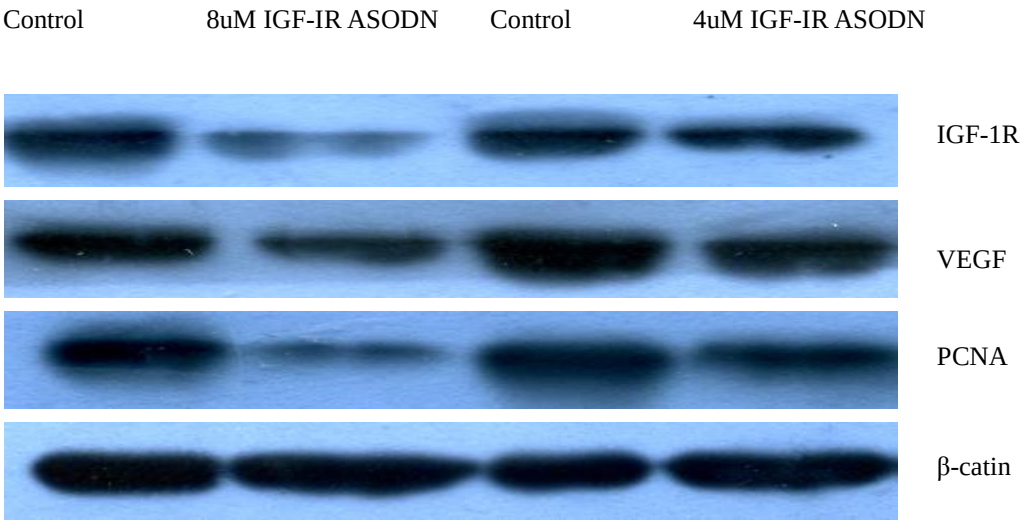

Figure 1. Western blot detecting expression of IGF-IR, VEGF and PCNA after IGF-IR ASODN transfected MDA-MB-231 breast cancer cells

2.5. Western blotting methods

Single cell suspension was diluted into 2.5×105 /mL and implanted into 6 well plates, 2 mL for each well. The suspends are cultured for 24 hours, cultured in no serum culture medium for 24 hours after cells creep plates and added 8μmol/L IGF-IR ASODN. After finishing of the above steps, cell lysate was added for schizolysis and cell total protein was extracted. S-PAGE electrophoresis was finished with Bradford method. Put extracted protein on PVDF membrane and occluded the membrane with 5% skim milk at room temperature for 1 hour. After combined with relative an antibody, the protein was incubated in 4 ℃ for one night. And then the total protein was combined with HRP marked secondary antibody and kept in table concentrator in 37℃ for one hour. After colouration with ECL, sensitization was finished with X-ray film taken in the darkroom. Developing and fixing X-ray film at room temperature. Integrated optical density (IOD) was detected with scanner and Gelation Image master. β-actin protein bands was taken as internal standard. Expression of Ki-67, VEGF and proliferating cell nuclear antigen (PCNA) were detected by immunohistochemistry.

2.6. MTT testing cell proliferation conditions of breast cancer cells

Cells in logistic period were chosen, digested and made into 1.0×106 /ml cell suspends. Cell suspends were implanted into 96-wells plates (100μl for each well) and stored for 6hours. Complete medium was suctioned when adhesion of cells and conditioned medium was added. The cells were maintained for 6 hours and added IGF-IR ASODN with 2, 4, 8μmol/L. The blank control group did not add any nucleic acid. Add 20μl MTT（5mg/L）24, 46, 72 and 96hs later and cell suspension was kept for 4 hours. The culture fluid was suctioned while 150μl DMSO was added. 6 hours later, absorbency (A) of each well were tested with ELISA (3 wells for each group). Calculated out cell proliferation inhibition rate: (1-A of experimental group/A of blank control group) ×100%.

2.7. Statistical methods

Data in the study were calculated out according to SPSS13.0 software. The relationship between IGF-IR and Cell proliferation was analyzed with t test. It has significance when the value of P is lower than 0.05.

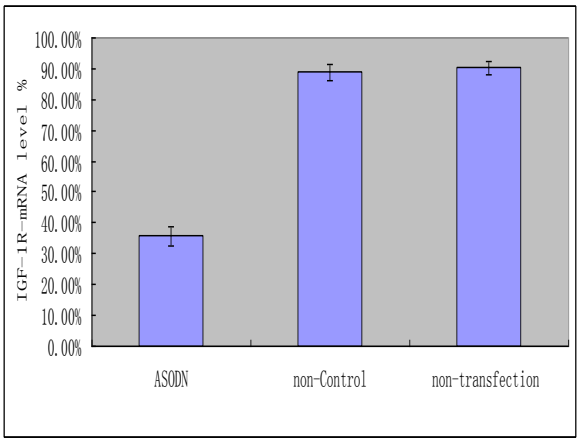

Figure 2. RT-PCR methods detecting expression of IGF-IR mRNA after 8uM IGF-IR ASODN transfected MDA-MB-231 in breast cancer cells.

3. Results

3.1. WESTERN methods detecting expression of IGF-IR, VEGF and PCNA after IGF-IR ASODN transfected MDA-MB-231 breast cancer cells

Before transfection, IGF-IR is highly expressed in MDA-MB-231 breast cancer cells. After transfection, IGF-IR expression is inhibited. It is also true for expression of VEGF and PCNA (Figure1).

3.2. RT-PCR methods detecting expression of IGF-IR, VEGF and PCNA after IGF-IR ASODN transfected MDA-MB-231 breast cancer cells

After transfection, expression rate of IGF-IR mRNA is lowered to 35.77% while the expression rate of IGF-IR mRNA of no transfection group and blank control group are as high as 88.94% and 90.24% respectively (Figure 2).

3.3. Results of application of MTT in different concentration

After transfected with IGF-IR ASODN, 2-8uM IGF-IR ASODN, it can inhibit expression of IGF-IR in some way and inhibit breast cancer cell proliferation in vitro (Figure 3, table 1).

Table 1 Comparison of cell proliferation inhibition rate tested by MTT before and after transfection.

Group	Inhibition rate(%)		
	24h	48h	72h
2 umol/L	23.54±2.36	32.47±3.02	29.24±0.24
4 umol/L	29.66±3.64	49.32±4.29	33.06±2.74
8 umol/L	51.32±4.21	68.23±3.33	58.64±5.01
Blank control group	5.14±0.47	8.98±1.02	7.24±0.52
No transfection group	6.33±0.55	10.51±0.67	8.23±0.41

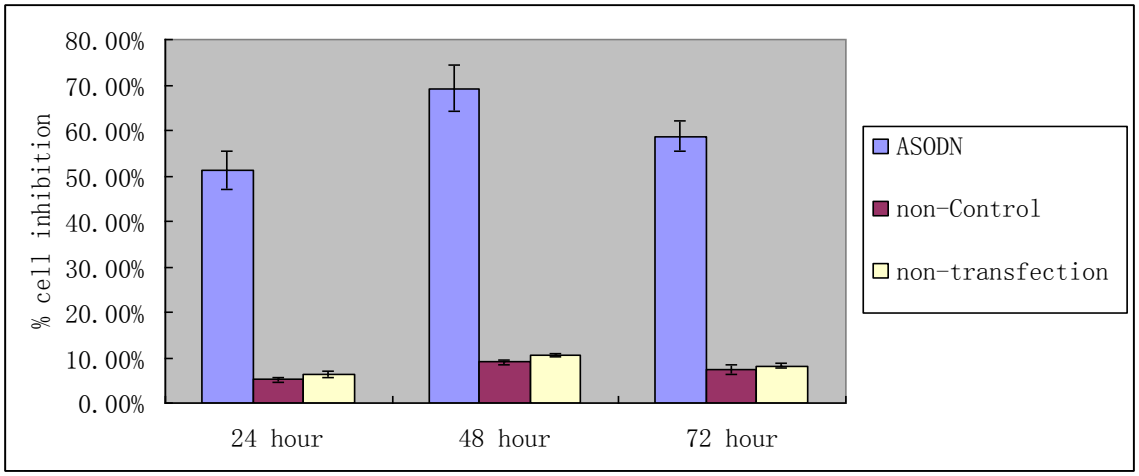

Figure 3. After transfected with 8uM IGF-IR ASODN, cell proliferation inhibition rate isobviously increased.

4. Discussions

Biological function of IGF-IR has made it an important target gene in tumor treatment and inhibiting and activating expression of IGF-IR with IGF-IR ASODN, micromolecule tyrosine kinase inhibitor, inhibiting antibody, dominant negative mutant and many other ways have been studied by many tumor studies and zoopery [8-10]. Some of the studies have got a good resolution of inhibiting expression of IGF-IR and have made further clinical studies [11-13]. AS a gene therapy method, IGF-IR ASODN have got brilliant result in treating cancers and have been applied in treatment of melanoma, Osteosarcoma, cutaneous cancer, colon cancer, glioma, breast cancer and many other cancers and IGF-I and IGF-IR

ASODN which have obtained inhibition effect both in vivo and vitro [14-16]. They concluded that it is more useful to treat cancer with focus on IGF-IR receptor than IGF-IR ligand [17-18]. The relationship between IGF-IR and onset and development of breast cancer has been confirmed in the first part in our study, and it is enough to confirm that IGF-IR can be index to diagnose and determine TNM period of breast cancer and target gene for gene therapy and molecule drugs. IGF-IR can be widely developed and used in pharmaceutical area. Our study further researched inhibition of IGF-IR ASODN on inhibiting expression of IGF-IR in breast cancer cells in vitro after transfected in breast cancer cells. This study proves that IGF-IR is highly expressed in breast cancer MDA-MB-231 cells, which confirm that it is right to choose MDA-MB-231 cells to finish the study about IGF-IR. This study uses MTT method to detect influence of IGF-IR ASODN on growth and proliferation of breast cancer MDA-MB-231cells. The result shows that IGF-IR ASODN can markedly inhibit growth and proliferation of cells and inhibition effect increases while the concentration of IGF-IR increase. Inhibition effect on breast cancer MDA-MB-231 cells also increases with length of time. With the low expression of IGF-IR, expression of VEGF and PCNA also lowered. Angiogenesis is an important link of tumor growth invasiveness and metastasis. Thus angiogenesis factors are very important in tumor occurrence and metastasis and target factor of inhibiting tumor angiogenesis [19-20].

In this study, IGF-IR ASODN can inhibit expression of VEGF which proves that IGF-IR ASODN can obviously inhibit angiogenesis.

In brief, autocrine and paracrine of IGF-I induced by IGF-IR are very important in growth of breast tumor and angiogenesis. Treatment with IGF-IR ASODN can effectively inhibit proliferation of breast cancer cells and angiogenesis ability.

Acknowledgement

We thanks our study was supported by Guangdong Science and Technology Applied Basic Research Projects, number: 2013 j4100059.

References

[1] Cecil DL, Park KH, Gad E, Childs JS, Higgins DM, Plymate SR. T-helper I immunity, specific for the breast cancer antigen insulin-like growth factor-I receptor (IGF-IR), is associated with increased adiposity. Breast Cancer Res. Treat, 139(3) 2013 657-665.

[2] Sayeed A, Fedele C, Trerotola M, Ganguly KK, Languino LR. IGF-IR promotes prostate cancer growth by stabilizing α5β1 integrin protein levels. PLoS One, 8(10) 2013 76513.

[3] Fox EM, Kuba MG, Miller TW, Davies BR, Arteaga CL. Autocrine IGF-I/insulin receptor axis compensates for inhibition of AKT in ER-positive breast cancercells with resistance to estrogen deprivation. Breast Cancer Res, 15(4) 2013 R55.

[4] Dean JP, Sprenger CC, Wan J, Haugk K, Ellis WJ, Lin DW. Response of the insulin-like growth factor (IGF) system to IGF-IR inhibition and androgen deprivation in a neoadjuvant prostate cancer trial: effects of obesity and androgen deprivation. J. Clin. Endocrinol Metab, 98(5) 2013, E820-828.

[5] Alawad AA. Evaluation of clinical and pathological response after two cycles of neoadjuvant chemotherapy on sudanese patients with locally advanced breast cancer. Ethiop J. Health Sci., 24(1) 2014 15-20.

[6] Singh P, Alex JM, Bast F. Insulin receptor (IR) and insulin-like growth factor receptor 1 (IGF-1R) signaling systems: novel treatment strategies for cancer. Med. Oncol, 31(1) 2014 805.

[7] Cern á M, Narsansk á A, Treska V, Kucera R, Topolcan O. IGF1 and tumor markers in different breast cancer stages. Rozhl Chir., 90(12) 2011 688-694.

[8] Gallagher EJ, Alikhani N, Tobin-Hess A, Blank J, Buffin NJ, Zelenko Z. Insulin receptor phosphorylation by endogenous insulin or the insulin analog AspB10 promotes mammary tumor growth independent of the IGF-I receptor. Diabetes, 62(10) 2013 3553-3560.

[9] Sivaramakrishnan M, Kashyap AS, Amrein B, Saenger S, Meier S, Staudenmaier C. PEGylation of lysine residues reduces the pro-migratory activity of IGF-I. Biochim Biophys Acta, 1830(10) 2013 4734-4742.

[10] Razmkhah M, Jaberipour M, Hosseini A, Safaei A, Khalatbari B, Ghaderi A. Expression profile of IL-8 and growth factors in breast cancer cells and adipose-derived stem cells (ASCs) isolated from breast carcinoma. Cell Immunol, 265(1) 2010 80-85.

[11] Majo VJ, Arango V, Simpson NR, Prabhakaran J, Kassir SA, Underwood MD. Synthesis and in vitro evaluation of [18F]BMS-754807: a potential PET ligand for IGF-1R. Bioorg. Med. Chem. Lett., 23(14) 2013 4191-4194.

[12] Happerfield LC, Miles DW, Barnes MD. The loeatization of the Insulin-like growth faetor 1 in benign and malignant breast tissue. J. Pathol., 183 1997 412-417.

[13] Mauro L, Pellegrino M, De Amicis F, Ricchio E, Giordano F, Rizza P. Evidences that estrogen receptor α interferes with adiponectin effects on breast cancer cell growth. Cell Cycle, 13(4) 2014 553-564.

[14] Caixeiro NJ, Martin JL, Scott CD. Silencing

the mannose 6-phosphate/IGF-II receptor differentially affects tumorigenic properties of normal breast epithelial cells. Int. J. Cancer, 133(11) 2013 2542-2550.

[15] Hermani A, Shukla A, Medunjanin S, Werner H, Mayer D. Insulin-like growth factor binding protein-4 and -5 modulate ligand-dependent estrogen receptor-α activation in breast cancer cells in an IGF-independent manner. Cell Signal, 25(6) 2013 1395-1402.

[16] Goel S, Gupta N, Walcott BP, Snuderl M, Kesler CT, Kirkpatrick ND,et al.Effects of vascular-endothelial protein tyrosine phosphatase inhibition on breast cancer vasculature and metastatic progression[J].J Natl Cancer Inst, 105(16) 2013 1188-201.

[17] Li X, Feng Y, Liu J, Feng X, Zhou K, Tang X. Epigallocatechin-3-gallate inhibits IGF-I-stimulated lung cancer angiogenesis through downregulation of HIF-1α and VEGF expression. J. Nutrigenet Nutrigenomics, 6(3) 2013 169-178.

[18] Olmos A, Díaz L, Avila E, Barrera D, López-Marure R, Biruete B. Associations between insulin-like growth factor I, vascular endothelial growth factor and its soluble receptor 1 in umbilical serum and endothelial cells obtained from normotensive and preeclamptic pregnancies. Growth Factors, 31(4) 2013 123-129.

[19] Xie M, He G, Wang R, Shi S, Chen J, Ye Y. Matrine-induced Apoptosis of Human Nasopharyngeal Carcinoma Cells via In Vitro Vascular Endothelial Growth Factor-A/Extracellular Signal-regulated Kinase1/2 Pathway Inactivation. Horm Metab Res., 2014.

[20] Hu J, Su P, Jia M, Wu X, Zhang H, Li W. TRPS1 expression promotes angiogenesis and affects VEGFA expression in breast cancer. Exp. Biol. Med. (Maywood), 2014.