The Effect of Kanglaite Injection on Expression of MUC1 MUCIN and Its Biological Meaning

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Abstract

Purposes: To study the effect of KLT of the expression of MUC1 mucin and the molecular mechanism of its antitumor action. Methods: To detect the effect of KLT on the expression of MUC1 gene、protein by using quantitative RT-PCR analysis, and flow cytometry instrument.

Results: 1. The results of RT-PCR demonstrated that after treated by KLT (15 μl /ml) for 72hr, the expression level of MUC1 gene decreased to 0.244 from 0.849 of drug-free group. 2. Analysis of flow cytometry showed 5μl/ml of KLT treat MC F7 cells for 7 days, the expression level of MUC1 protein was unchanged, whereas 10μl/ml of KLT treating MCF7 cells for 5-7days, the expression level of cell surface MUC1 molecules decreased to 69.6% from 91.0% of drug-free group, their difference was significantly.

Conclusion: KLT could make MUC1 gene and protein of MCF7 cells under expression, which was dose and time dependent. This down expression might play an important role in the antitumor metastasis of KLT.

KLT, a new type of traditional Chinese medicine, is effective against a variety of tumors in clinical and laboratory studies. Carcinogenesis is a process containing a number of steps and factors, in order to treat cancer patients effectively, it is necessary to control and block various tumor-related segments which results in delaying and eliminating tumors. Studying the mechanism of anti-tumor action of KLT from different aspects is needed. MUNI mucin (designated MUCI) is a transmembrane glycoprotein with aberrant and over expression in epithelial cancers, which is highly related to invasion、metastasis and prognosis of tumors and also takes part to signal transduction of tumor cells. In present paper, the effect of KLT on the expression of MUCI gene and its protein products in mammary cancer cell line in vitro was studied and its biological mean of these changes was also explored.

1. Materials and Methods

1.1 Cancer Cell Line

Human mammary cancer cell line MCF7, reserved in our laboratory

1.2 Drugs and Reagents

KLT and emulsion provided by Kanglaite pharmaceutical CO. Ltd.(Zhejiang PRC). 3(4, 5-dimethyl-2 thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT, sigma product)

guanidinium thiocyanate、RNacin、AMV reverse transcriptase (Promega CO. products), RPM11640, bovine serum (Gibco, USA), Taq enzyme and related reagents(Tabara products). MUCI monoclonal antibody BC2 was generously provided by Dr. Xin Pei Xiang (Australia) IgG/FITC (Beijing Zhongshan biological technique CO. product)

1.3 PCR Primer Design

MUC1 and β-macroglobulin primers were prepared by the method reported in reference 3.4. The length of fragment amplified was 288bp and 150bp respectively (synthesized by Shanghai Shenggong biological engineer CO.)

1.4 Instrument

PCR instrument is PE 2400 Type. Flow cytometry (BD. CO. FACS440).

1.5 Methods

1.5.1 Drug Sensitive Test of Cells-MTT Method

Take exponential growth phase MCF7 cells, digested by pepsin, adjusted the cell concentration to $3\text{-}5\times10^4$/ml. Inoculated 0.1ml of cell into 96-aperture Millipore microculture plate and incubated for 24 hr. Various concentration of antitumor drugs were added to each pore, 3 pores for each drug concentration, continued to incubate for 3 days. Discarding the culture medium, MTT 0.4/ml 200μl were added to each pore, incubated 4h, discard the MTT solution, add DMSO 150μl to each pore, till the crystal dissolved completely, and then measure the absorptance for each pore with enzyme labeling photometer Anthos 2010 at wave length 570nm and calculate the half inhibitory concentration(IC_{50}).

1.5.2 Cellular Total RNA Extraction

MCF7 cells $3\text{-}5\times10^5$ were inoculated into each flask, incubated for 24hr to enter logarithmic growth phase. In tested groups various final concentrations of KLT 5、10、15 and 20μl/ml were added respectively, and drug-free emulsion regarded as control group incubated for 72hrs, digested by pepsin, washed with PBS and centrifuged. One step method of extracting RNA by using guanidinium thiocyanate-phenol-chloroform.

1.5.3 RT-PCR

Cellular total RNA 1μg and mixture containing 5xRT buffer solution 4μl, oligo (dT) 10μg/ml and dNTP 0.5mol/L, predenatured for 5 min at 65℃. Then left in ice bath for 3min, added RNasin 10U、AMV reverse transcriptase 10U, for 60min at 42℃, for 5 min at 95℃. 2μl of 10x PCR buffer solution, 10pmol/L of both MUC1 and β-macroglobulin primer, and taq DNA polymerase 1w(total volume 20μl were added to 2μl of above reverse transcription products to perform PCR amplification. Cycling parameters was predenatured 5 min at 95℃, denatured for 45sec at 95℃, appealed 45sec at 65℃, prolonged 1 min at 72℃, total 35 cycles.

1.5.4 Quantitative Analysis of PCR Products

Take 10μl of amplification products to run a 2.0% agarose gel electrophoresis and stained with ethidium bromide, then photographed by using kodak Digital science gel imaging system. To analyze the amount of each band quantitatively by ID soft ware and calculate the ratio of MUC1 and β-macroglobulin.

1.5.5 Detestations of Membrane Surface Molecules-Cell Flow Cytometry

Inoculate 3-5×10^5 cells for each bottle, incubate for 24hr whereas the cells growing in logarithmic phase. Tested groups were treated with 5μg/ml, 10μl/ml of KLT (final concentration) respectively, and incubated in 5% CO_2 incubator, at 37℃for 5 and 7 days, digested with pepsin and added MUC1、monoclonal antibody BC_2 respectively. Normal mice serum was added to blank control group, in addition, emulsion control group was set up. Each tube was added inactivated normal rabbit serum 50μl, kept at 4℃for 30min, washed with PBS twice, added IgG/FITC for 30min at 4℃, again washed with PBS twice fixed with 4% polyformaldehyde for flow cytometry assay.

2. Results

2.1 Drug Sensitive Test in MCF7

IC_{50} of KLT on MCF cells was 19.03μl/ml.

2.2 The Effect of KLT on Expression of MUC1 Gene

After treated with various concentration of KLT (5, 10, 15μg/ml) for 72hr, the expression of MUC1 gene in MCF7 cells was carried out by using quantitative RT-PCR process. As shown in Fig1, when KLT concentration increased gradually, the expression level of MUCI gene decreased to 0.244 from 0.849 of drug-free group. It indicated that KLT could reduce the expression of MUC1 gene markedly in MCF7 cells.

2.3 The Effect of KLT on Expression of MUC1 Protein

The results of cell flow cytometry analysis demonstrated that the expression of MUC1 protein in MCF7 cells untreated with emulsion was 96.3%, in treated with emulsion for 5 days was 89.5%. There was no significant difference existed. MCF-7 cells treated with 5μg/ml of KLT for 5 days, the expression level of MUC1 was no markedly changed, but with 10μg/ml of KLT for 5 or 7 days, the expression level of cell surface MUC1 molecules decreased to 69.6% and 53.1% respectively instead of 91.0% of drug-free group (Fig 2). Thus, KLT could down regulate the expression of MUC1 protein of MCF7 cells, and was dose-, time- dependent.

3. Discussion

MUC1 is one kind of type I transmembrane glycoprotein which is mainly disturbed at the luminal side of glandular epithelial cells of mammary gland, ovary, pancreases, colon, lung and stomach etc and presented in apical and polarized expression. After cell carcinogenesis,

the property and level of MUCI changed a lot, showed as follows: (1) the level of expression can increased more than 10-fold as compared to their normal counterparts; (2) polarized distribution of cell surface molecule is lost; (3)the structure was changed, new peptide chain and glucochain phenotype appeared.

Recently, MUC1 has been used to diagnose and treat a number of cancers in which MUC1 R1A could be regarded as the marker of early detection of phase II/III advanced mammary cancer and has been approved by FDA of America in 1997. MUC1 is a antigen molecule which is highly related with the genesis and development of tumors. Studying the effect of KLT on the expression of MUC1 would be helpful to explore the molecular mode of its antitumor action.

Our experiments showed that KLT could reduce the expression of both MUC1 gene and MUC1 protein, its mechanism might be mediated the inhibition of MUC1 gene transcription, and further the synthesis of protein. This kind of down regulation of expression may be useful for cancer treatment. MUCI is also related to tumor invasion and metastatic which was demonstrated by our results. At first, MUC1 could down-regulate the expression of E-cadherin, which is a Ca^{2+}-dependent cell adhesion molecule, mediated cell-cell interactions and inhibit tumor metastatic. The down-regulating of expression by E-cadherin could potent the invasive activity of tumor cells. Second, the high density of expression of rigid MUC1 molecules which located on the tumor cell membrane could hamper the interaction of ligands of membrane surface with their receptors and also able to reduce integrin-mediated extracellular matrix interactions; in addition, sialyl-Lewisx domain of MUC1, regarding as ligands of E-selectin, could reacted with E-selectin which located on the damaged or imflammative blood vessel endothelial cells. The outcome of above reactions makes tumor easily cells adhesion with endothelial cells and permeate the vessel wall, causing tumor cell metastasis from blood stream. In brief, high level expression of MUC1 could stimulate tumor metastasis. Thus, it is possible that KLT decreased the expression level of MUC1, regulated the interaction of tumor cells, stromatic cells with endothelial cells of blood vessel, and then inhibited the cancer metastasis. This is in accordance with the result of antitumor metastasis of KLT in clinical and laboratory studies.

Besides, MUC1 is related with signal transduction of tumor cells. Tyrosine (Y) of endocellular YTNP domain could be phosphorylated and could bind with the function site of adaptor protein Grb2. Grb2 binds to the guanine nucleotide-releasing factor SOS through its SH3 domains resulting in the formation of a MUC1/Grb2/SOS complex. SOS binds to the Ras at the cell membrane, supporting a role for MUC1 in signal transduction of ras pathway. Thus, KLT might mediate the down-regulation of MUC1 to modulate the intracellular signaling of tumor cells.