Anticancer effect of the IgY that produced against a small peptide with 15 amino acids of human DR5 on MCF7 cell line

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ABSTRACT

Tumor necrosis factor- α (TNF-α) plays an important role in diverse cellular events such as septic shock, induction of other cytokines, cell proliferation and apoptosis. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is currently attracting great interest as a potential anticancer drug. TRAIL could selectively induce apoptosis in tumor cells in vitro and in vivo by a death receptor-mediated process. TRAIL shows a high degree of promiscuity as it binds to the DR5 receptor and it is generating considerable interests as a possible anticancer therapeutic agent. Use of TRAIL or its antagonist could be a good anticancer treatment in future. The extracellular domain of DR5 human protein which has the attachment part of this ligand to TRAIL ligand is considerable domain of it. We produced a small peptide with just 15 aminoacids from this domain, with peptide synthesizer. Then inject them to hens to immunize them and achieve high affinity IgY. At least, obtained IgYs specially recognize DR5 protein and in vitro start exclusively to induce death in the MCF7 cell line, and interestingly not on normal cells.

Keywords: Cancer; Yolk Immunoglobulin; Chicken; DR5.

INTRODUCTION

Nowadays cancer is the second cause of death among human. If a branch of cells could not remove the cells with genetical damages, beyond more and more amplification of them, they go out of control and cancerous cells birth. In the other words, the cells forget the dying. Cancer is subsequent of series of mutations in growth, cell cycle control, apoptosis inducing and oncogene genes. Furthermore these mutations disrupt the natural process of the cells [1,2]. Ligation of death receptors of the tumor necrosis factor receptor super family such as CD95 (APO-1/Fas) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors by their corresponding natural ligands results in the recruitment of caspase-8 into multimeric complex at the plasma membrane, the death inducing signaling complex (DISC) [3].

Among these receptors, it seems TRAIL receptors have more specificity for cancerous cells [4-7]).

TRAIL shows a high degree of promising as it binds to five cognate receptors: DR4 (TRAIL-R1) and DR5 (TRAIL-R2) and the decoy receptors DcR1 (TRAIL-R3), DcR2 (TRAIL-R4), and osteoprotegerin (OPG) [8]. DR4 or DR5 receptor-specific TRAIL variants would permit new and tumor selective therapies. DR5 was described as contributing more than DR4 to the overall apoptotic activity of TRAIL in apoptosis signaling of cancer cells [9-11].

Production of agonists of TRAIL like antibodies can lead to activation of these receptors. From two kinds of poly- and monoclonal antibodies, the major problem of monoclonal antibody is that some antigens are weakly or not at all immunogenic for animals [12-15].

During the past 20 years, the use of chickens against of mammals for antibody production has increased. A major advantage of using birds is that the antibodies can be harvested from the egg yolk instead of serum, thus they did not hurt animals. Additionally, antibody productivity of an egg-laying hens is much greater than that of a similar sized mammal [16]. The development of lipid based drug carrier has attracted increased attention over the last years. This lipid carriers are rapidly developing field of nanotechnology with several potential application in drug delivery clinical medicine and research, adjuvant [17]. Protection of incorporated active compounds against chemichal and enzymatical degradation and more flexibly in modulating the release of the compound, they are composed [18].
The combination of such advantages, occupying the death receptor with an antibody that produced in birds, can give a strong weapon against MCF7 cancerous cells. In this study we choose a small part of the extracellular domain of the DR5-receptor, synthesized it and used for immunization of the hens. At least evaluate its effect on breast cancer Cells.

MATERIALS AND METHODS

Peptide production:

A 15 amino acids peptide is produced in peptide synthesis center of National Institute of Genetic Engineering & Biotechnology.

Liposome prepration:

Liposome were prepared by the dehydration/rehydreation process. Lipids (lesitin, 500 mg, cholesterol 5 mg) was dissolved in ethanol (25 ml) and dried to film in a round-bottom flask using a rotator evaporator at 50^0C. The film was solved in sterile PBS (20 ml, Na_2HPO_4 5.8 gr, KH_2PO_4 1.63gr, NaCl 5.3 gr). The mixture was sonicated (600 herts, 5 minutes) and then added dextran (2.5 mg). Then the performed liposome were dehydrated by freeze-drying using a liophilizer. They were sealed and stored in a refrigerator at -20C.

Immunization of hens

Immunization of hens was performed in animal house of Pastour institute (Tehran, Iran) with a total 350µg of peptide which was encapsulated in liposomal structure.

ELISA

Ninety-six-well ELISA plates were coated O/N with 20µg of either recombinant synthesized peptide and incubate coating buffer for one over-night in 4^oC. The coating was followed by 60 min incubation with blocking buffer then incubate for 45 min with the tested IgY antibody (dilutions 1:100 to 1:1600 were used), then 30 min incubation with secondary antibodies (goat derived anti-hen-IgG) ,HRP conjugated. We have washing in every step for 3 times with PBST. In the detection step, add 100µl TMB solution and absorbance was measured at either 490 nm (reactions stopped with 1 N hydrochloric acid).

Isolation and purification of IgY:

Isolation of IgY was carried out by separation of the egg yolk from the white part. The egg yolk was added two volumes of phosphate buffer (sodium phosphate 0.1M, NaCl 10mM, pH=7). The solution was then mixed with magnetic stirrer. Then add two volume chloroform and incubate in 4^0C for 20 minutes. Centrifuge the solution in 14000 ×g, for 20 minute, collect the supernatant and discard the plate. 12% (w/v) poly ethylene glycol (PEG6000) was added to the supernatant and mixed with magnetic stirrer for 20 minutes to remove lipoproteins. The mixture was centrifuged at 14000 ×g, 10 minute at 4^oC and the supernatant was discard and sediment resolved in PBS (PH=7.4). For more purification of IgY repeat the last step three. At least the sediment was then resuspended in equal volume of phosphate buffer and preserved at 4^oC until further use.

MTT assay:

The MTT method of cell determination is done in three days on MCF7 cell line (human breast cancer). First cultured 10000cells per well in the first day and incubate it for 24 hours. In the second day, remove media carefully and treat cells with against, antibodies (final volume should be 100 µl). Day three remove the supernatant and add 100 µl of 5 mg/ml MTT to each well. Incubate them for 2 hours at 37^0C, 5% CO_2 in culture incubator. Carefully remove media and resolve the color in MTT solvent (isopropanol). Read the absorbance at 590 nm. Then calculate the data with Farms software.

RESULTS

Nanoliposome prepration

Produced liposomal compositions were in a nano scale. However, liposomal size was meseared with Zetasizer nano. The ranges of liposomal suspension for liposome without cargo were 11.7 nm and for liposoms with peptides were 73.5 nm (figure 1). The obtained milky liquid of liposomal constructions encapsulated 1 mg/ml of 15 aminoacids peptide. Indeed, the differences between free and loaded liposome configure the encapsulation.

IgY production

ELISA assay was performed to stimulate antibody production and its specificity. Furthermore, after adding the purified IgYs to the coated wells, they attach remarkably to the peptides (figure 2). According to the small size of the injected peptides the amount of the antibody titer was significant.

MTT results

To estimating the ability of IgY-15L to kill the MCF7 cancerous cells, MTT assay was used. The result showed that IgY-15L with 2.5 µg/ml kill the 50% of MCF7 cells selectively. According to the specific interaction of IgY-15Lwith DR5 receptor, that seems it can induces the death cascade in these cells (figure 3).

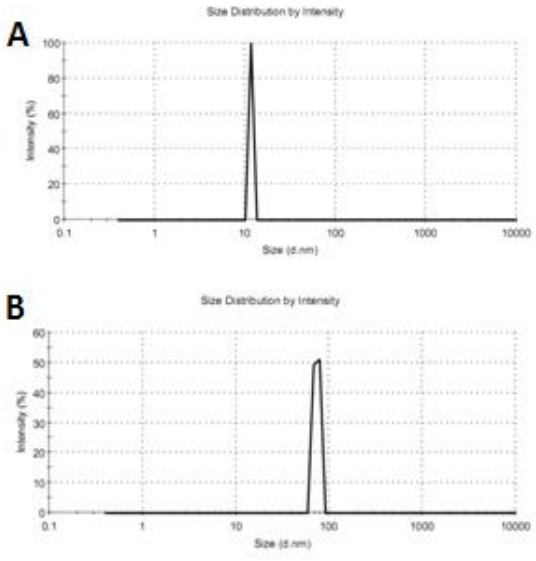

Figure 1. A histogram of liposom size distribution determined by DLS. The diameter shown is the hydrodynamic diameter of the free liposome (A) and liposomes that were loaded with peptides (B).

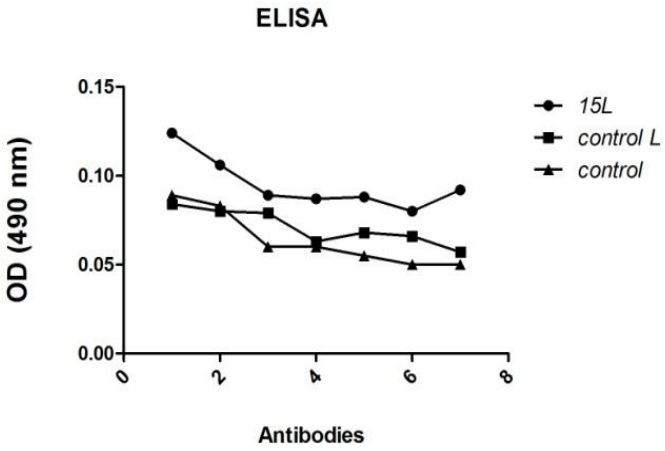

Figure 2. ELISA assay result of IgY that produced against 15 aminoacids of extracellular part of DR5 protein.15L is antibody that produced against nanoliposomal 15 amino acids peptide, control L is antibody against just free nanoliposom and control is IgY that purified from nonimmunized hens.

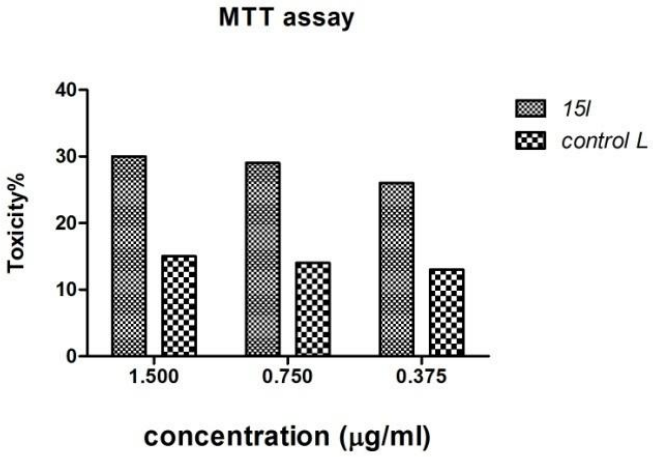

Figure 3. MTT assay results. The result toxicity per cent of IgGs (15L and control L) on MCF7 cell line. The means differences for $P<0.05$ has sense. The percentage of toxicity was calculated using the following formula: %toxicity= (AT/AUT – 1)×100, where AT is absorbance of treated cells, and AUT is absobance of untreated cells.

DISCUSSION

In recent years, a surge in our knowledge about programmed cell death (apoptosis) has established its role in the regulation of tumorigenesis. Apoptosis is an active, energy-dependent process that mediates the elimination of cells that have developed improperly, or sustained irreparable genetic damage [19]. At the end point we started

apoptosis process in an another properly substitute pathway upon dead receptors. It seems death receptors are a good one. TRAIL is a 32 kDa transmembrane protein expressed in a wide range of normal fetal and adult tissues, suggesting the existence of a protective mechanism against its cytotoxicity in normal cells. This is supported by observations that TRAIL can induce apoptosis in transformed and malignant cells, but not in normal cells. TRAIL induces apoptosis by interacting with two cell-surface receptors, DR4 and DR5 [20]. The selective nature of the anticancer activity of TRAIL was demonstrated by studies in mice and non-human primates [21,22].

After Klemperer discovery that the immunization of hens results in transfering the specific IgY from serum to eggs till now, the Scientifics produce several kinds of IgYs that can stop or control their target pathological organisms [23,24]. The use of chicken IgY, instead of IgG mammalian antibodies, to detect non-self or even self antigens, certainly may help lower costs of clinical or research immunological tests. In addition, chicken antibodies do not activate the mammalian complement system nor interact with rheumatoid factors, or bacterial and human Fc receptors. It seems using the antibody produced by hens to attaching this receptor and mimicking its work can be efficient. As we see, the antibody against a small part of the extracellular domain of DR5 receptor could specifically attach and induce its activation.

CONCLUSION

The present study introduce a functional and efficient new agent in cancer therapy. Such specific antibody could find cancer cells and kill them. However, they can used in drug delivery extensively.

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