Review Article

An overview of purification, biological activities and therapeutic applications of thymosin alpha

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Keywords:
Immunostimulatory, Thymus, Antitumor, Cellular Protection, Infectious Disease

Funding
None

Competing Interest
None declared.

Received: July 21, 2012
Accepted: September 24, 2012

Abstract

Thymosin alpha 1 (Tα1) is 28 amino acid residues peptide. Thymic epithelial cells produce acetylated N-terminal Tα1 that has powerful immunostimulatory and antitumor activites. Inside the body Tα1 effects endocrine, immune and central nervous system. Tα1 can be purified by using fractionation procedures from thymus. Many recombinant DNA techniques are being used for the production of Tα1. In addition Tα1 is being expressed as fusion proteins with other therapeutically important proteins. For the high-throughput production of Tα1, different expression systems are being used such as *E.coli* and yeast. Tα1 has unique antitumor and immunoregulatory properties and also have capacity to protect cells from oxidative damage. Tα1 has many therapeutic applications specifically against many infectious diseases (hepatitis B&C, AIDS, SARS etc.) and cancer.

Introduction

Thymosin alpha 1 was first isolated from calf thymus.[1] It's a small peptide of 28 amino acids with a molecular weight of 3KDa.[2] Thymic epithelial cells produce acetylated N-terminal Thymosin alpha 1 and it's stable at 80–90 °C.[3] Goldstein and Badamchian explained the multiple actions of thymosin on endocrine, immune, and central nervous systems (CNS).[4] Thymosin fraction 5 (TF5) was isolated on further purification of thymosin and this thymosin fraction 5 is heat stable and acetone insoluble. TF5 plays important role in inducing apoptosis of neuroendocrine tumor cells[5], enhancing immunological function and induction of T cell differentiation.[6] Amazing results seen with TF5 urged scientists to investigate further the factor present in TF5 which is involved in reconstitution of T-cell immunity. Due to this investigation Tα1 was purified from TF5.[7] Tα1 has been found 10 to 1000 times more active than TF5 both *in vivo* and *in vitro*.[8]

Pro-thymosin α (ProTα) is a 109 amino acids acidic nuclear protein and it produces Tα1 on cleavage by asparaginyl endopeptidase.[9] Tα1 secretion has no modulators.[10] Despite its main presence in thymic epithelial cells, Tα1 is also present in both lymphoid and non-lymphoid tissues such as spleen, liver, kidney, lungs and brain.[11]

Isolation and purification of Thymosin

Many methods are being optimized and modified for the production and purification of Tα1 as its therapeutic applications are increasing day by day.[12]

Direct isolation and purification from thymus

Tα1 can be purified from bovine thymus tissue by using different fractionation procedures. The purification of Tα1 during fractionation is based upon the increase in activity per mg of protein by the rosette assay of Bach et al.[13]

Expression and purification of recombinant thymosin

Many recombinant DNA techniques are being used for the production of Tα1 and this is being expressed as fusion proteins also.

a. Expression and purification of Tα1 in E. coli

For the high-throughput production of Tα1, different expression systems can be used. Synthesized gene of human thymosin alpha 1 (Tα1) can be inserted into vectors like pET-28a, pET-9c, pThioHis B, pGEX-2T or pBV222 and then induced and expressed in strains of *E. coli*. Best expression level has been provided by BL21/pET-28a system that was almost 70% of the total bacterial protein. Protein can be visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein can also be monitored by mea-

ISSN (print): 2218-3299
ISSN (online): 2219-8083

suring the UV absorbance at 215 nm.[14]

The expressed protein can be purified by using nickel affinity chromatography. Expression level of protein can be estimated by the SDS-PAGE method.[15] Other methods for the separation and purification from *E. coli* are thermal denaturation and high performance liquid chromatography (HPLC).[16]

b. Expression of Tα1 in Yeast

For the construction of yeast expression system for Tα1, whole Tα1 DNA fragment can be amplified by PCR and then by using restriction enzymes, it can be cloned in pYES2 vector or other yeast vectors. This cloned vector can be transformed into yeast to induce the Tα1 expression. The protein can also be expressed in secretory form and can be purified from medium after pelleting the cells by centrifugation. The expressed protein can be estimated by western blots.[17] Recombinant Tα1 that is highly expressed by the yeast expression system can improve CD8+ level in immune inhibited mice.[17]

Monitoring of Tα1 in the body

In vitro, Tα1 can stimulate the proliferation of mouse splenic lymphocytes and it also increases the apoptosis in tumor cells.[18] *In vivo,* Tα1 can inhibit the tumor growth in B16 tumor-bearing mice.[14]

By using indirect immunofluorescence and peanut agglutinin (PNA) technique specified receptors for Tα1 on mouse thymocytes can be monitored.[19] Method of Lowry et al can be used for the estimation of protein concentrations.[20]

The biological activity of the thymic hormone can be assessed by a newly developed rosette assay, which permits measurement of thymus-dependent lymphoid cells.[13] Thymosin activity is associated with a physicochemical homogeneous protein of molecular weight 12,600. The hormonal activity is evident in *in vitro* incubation assay, after injection into adult thymectomized mice, and in prolonging survival of neonatally thymectomized mice and the reconstitution of their response to a skin allograft.[21]

Biological activities of Tα1

Antitumor

Tα1 has shown its effectiveness against different cancers both *in vivo* and *in vitro*. *In vitro* assays on SPC-A-1 lung adenocarcinoma and HepG2 human hepatoma cells has given proof of antitumor activity of Tα1.[22] Its subcutaneous injection into BALB/c-mice suppressed the lung and liver metastases.[23] In case of fisher rats, it decreased the incidence of carcinoma of mammary glands hence increasing the survival time.[24] Its anti-proliferative activity is at peak in case of small lung adenomas.[25] cDNA of human Tα1 and IFNω1 in dual-gene plasmid–liposome complex showed more anti-proliferative activity than singly used plasmid-liposome complex.[26] When used in conjunction with 5-FU, IL-2, Tα1 controls the tumor metastasis and enhances the rate of survival in case of DHD-K12 colorectal cancer model.[27]

Immunoregulation

The maturity of T cells is enhanced by Tα1.[28] It plays important role in differentiating precursor stem cells into $CD4^+$ and $CD8^+$ T cells.[29] Cells having viral infection are killed by cytotoxic lymphocytes ($CD8^+$ T cells) and natural killer cells.[30] It also triggers the activation of dendritic cells. In one case it exerted negative effect on tumor necrosis factor α and IL-1β which resulted in decrease of severe-ness of severe acute pancreatitis.[31]

The mechanism of action on immune system by thymosin is unclear. However, research work has shown that thymosin controls the expression of genes of MHC I, MHC II, different cytokines and immune regulators.[32] On exposure of thymosin, genes of different immune modulators like of chemokines, cytokines and major histocompatibility were upregulated.[33]

Safe guard from oxidative damage

A study showed that Tα1 weakens the effect of free radicals on liver tissues by influencing glutathione peroxidase (GSH-Px) and liver superoxidedismutase (SOD).[34] Tα1 induces the anti-oxidative ability in pancreatic tissues by increasing the activity of different enzymes such as catalase (CAT) and SOD.[35]

Central nervous system is also affected by Tα1.[36] Tα1 minimizes the effect of chemotherapeutical neurotoxicities when given in conjunction with chemotherapeutics.[37]

Therapeutical applications of Thymosin α 1

Thymosin is being used and clinical trials are going on for different disorders and diseases that include, respiratory distress syndrome, TB, lung infections peritonitis, acute cytomegalovirus infection, septic shock, severe acute respiratory syndrome, hepatitis B and C, AIDS etc.[11]

Applications against infectious disease

Hepatitis B

Chronic hepatitis B is distributed all over the world and has lethal affects such as cirrhosis and liver cancer.[38] Tα1 is been used as a therapy against hepatitis B due to its great antivir-

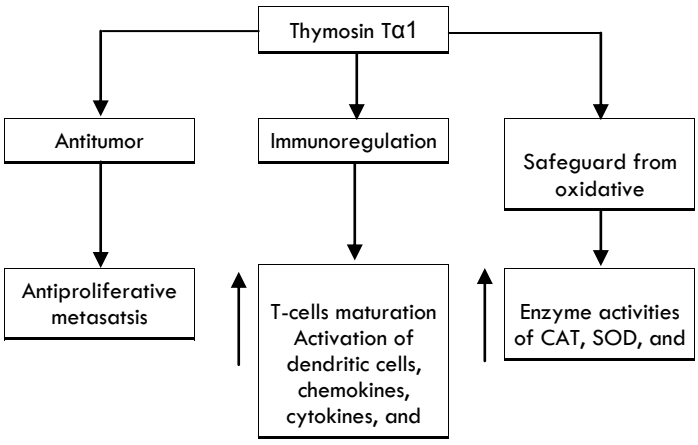

Figure 1: Different biological activities of Thymosin α1

al response.[39] In a clinical trial involving Chinese population, 1.6 mg dose of Tα1 exhibited normal values of alanine transaminase (ALT) in patients with HBeAg-negative chronic hepatitis B.[40] By materials from different data banks and meta analysis of eight trials Zhang et al showed that lamivudine in conjunction with Tα1 is more effective in terms of HBeAg seroconversion rate, antiviral response and normalization of ALT.[41] But a different group revealed opposite results[42] and this can be due to the small scale trial.

Hepatitis C

A double-blind, placebo-controlled trial showed that Tα1 is not a good choice for treating hepatitis C alone.[43] Combination therapy of Tα1 with pegylated interferon α2a (peg-IFN-α2a) reduced the viral replication sufficiently in patients who had difficulties in treating their hepatitis C with an advantage of having not too much side effects.[44] As many patients are non-responsive to standard ribavarin and peg-IFN therapy[45] so another combinational therapy including Tα1, ribavarin and peg-IFN-α2a has been devised which is safe.[46] It is an effective therapy for those patients who are non-responsive to conventional therapies.[47]

Acquired Immunodeficiency Syndrome (AIDS)

Cells having $CD4^+$ are the main targets of Human Immundeficiency Virus (HIV). After residing the lymphocytes HIV starts to destroy the $CD4^+$ T cells remarkably. This is followed by the decreased class switching of antibodies and less effective stimulation of $CD8^+$ T cells which accommodates the escalate of virus from immune components.[48] A strong immune system can prevent the infection by HIV so strong stimulation of immune system especially the CTL response can play a vital role in controlling the onset of AIDS.[49] One report showed that combinational therapy of zidovudine (AZT), IFN-α and Tα1 suppressed the HIV titers by increasing the $CD4^+$ numbers and function.[50] Chadwick et al determined the efficacy of Tα1 in conjunction with highly active antiretroviral therapy (HAART) for induction of strong immune response. Tα1 was tolerable and it enhanced the levels of signal joint T cell receptor excision circles (sjTREC) in patients with advanced HIV disease.[51]

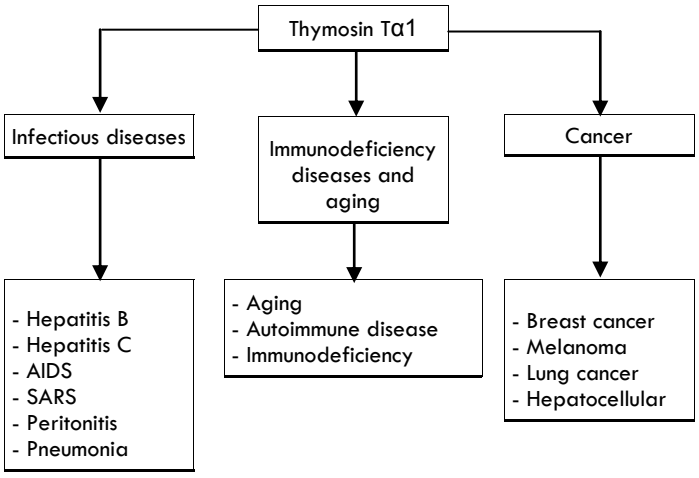

Figure 2: Different therapeutic applications of Thymosin α1

Severe Acute Respiratory Syndrome (SARS)

Tα1 prevents the spread of SARS that is caused by coronavirus and also controls the disease development. But the exact mechanism of Tα1 in prevention of SARS is not known yet.[11]

Acute respiratory distress syndrome (ARDS)

Tα1 has proved to repair cellular immunity by increasing the number of CD4+ and CD8+ lymphocytes. It increased the resistance against cytomegalo virus (CMV) that leads to ARDS. Tα1 has a quite high success rate to treat ARDS.[52]

Other Diseases

Tα1 is clinically proving to be very helpful in improving many other infectious diseases like severe sepsis, *Pseudomonas aeruginosa* pneumonia, severe chronic hepatitis, spontaneous peritonitis and help to restore immunity in many cancers like Lung cancer, melanoma, hepatocellular carcinoma. It has a role of chemoprotection during lung and breast cancers treatments and in many immune deficiencies like aging and immune senescence, autoimmune diseases etc.[11]

Tα1 is a very versatile protein with many different functions. It has influence on CNS and endocrine system in addition to immunoregulation. It has broad spectrum of biological activities so can be used for wide range of diseases in clinic whether alone or in combination with other therapeutics. It can be beneficial for those people who are suffering from chronic hepatitis B, hepatitis C, AIDS, etc. In addition it has enormous application to treat different cancer as it has great anti-cancer potential. Its bioactivity can be increased by using latest bioinformatics tools and genetic engineering.

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