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Cost-effective production of recombinant human interleukin 24 by lactose induction and a two-step denaturing and one-step refolding method

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Abstract Recombinant human interleukin 24 (rhIL24) is a member of the interleukin 10 (IL10) family of cytokines with novel therapeutic properties. Human IL24 possesses three *N* glycosylation sites and a disulfide bridge. The cost and composition of culture media is critical for commercial-scale production of recombinant proteins in *E. coli*. Addition of yeast extract and glucose to medium enhances rhIL24 production, and the use of lactose instead of IPTG for induction drops the cost and decreases toxicity. In addition, a two-step denaturing and one-step refolding (2DR) strategy improves rhIL24 production. The 2DR strategy replaces a more conventional approach for protein solubilization and refolding. LC–MS/MS provides definitive identification and quantitative information on rhIL24. Single-step purified rhIL24 displayed biological activity on HepG2 hepatocellular carcinoma cells, but no effect on L02 cells. Proliferation analysis suggests that rhIL24 may have potential use as a medication. In the present study, we developed a simple process for producing quality product with high purity. The expression and purification of rhIL24 described here may be a step towards inexpensive large-scale production.

Keywords rhIL24 · Cytokine · Lactose induction · LC–MS/MS · HepG2

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Introduction

Fisher identified melanoma differentiation-associated gene-7 (MDA-7) by subtractive hybridization. Upon discovery of MDA-7 as a cell surface receptor, it was renamed interleukin 24 (IL24). This gene's location, structure, and cytokine-like properties indicated that it was member of the interleukin 10 (IL10) family of cytokines [1]. Human IL24 possesses three consensus N glycosylation sites. Site 1 (Asn-85), Site II (Asn-99) and Site III (Asn-126) form a unique disulfide bridge between Cys-52 and Cys-126 [10]. Recombinant human interleukin 24 (rhIL24) has been expressed in *E. coli* as a fusion protein and as an adenovirus (AdV)-mediated protein [2], and presents several obstacles to achieving large-scale production. Fusion protein purification is time consuming and leads to a sharp increase in the cost of commercial-scale production [6]. Therefore, inexpensive and low toxicity production of non-fusion rhIL24 is important for functional study and characterization. Cost-effective and simple culture media is needed for commercial production of recombinant proteins in *E. coli* [8]. IPTG is routinely used to induce recombinant protein production but its toxicity increases production costs. Lactose is a less expensive and nontoxic alternative [11]. Interestingly, a two-step denaturing and one-step refolding strategy (2DR) leads to increased production of high purity recombinant protein, which minimizes purification efforts [23]. IL24 selectively restrains growth and induces

apoptosis in human cancer without significant side effects [18]. Hepatocellular carcinoma (HCC), which affects a number of patients but has a limited number of therapeutic options, has been the focus of recent research. HCC is the most common tumor in developing countries but is less frequent in Europe and the United States [7], resulting in thousands of deaths each year, while more than 50 % of cases are found in China [25].

The present study was designed to (1) evaluate the potential for lactose induction to replace IPTG, (2) understand the effect of a two-step denaturing and one-step refolding (2DR) strategy, and (3) assess the biological activity of rhIL24 with HepG2 cells.

Materials and methods

Bacterial strain and vector system

E. coli strain BL21 (DL3) (Novagen, Darmstadt, Germany) was used as a host for rhIL24 expression. BL21 was transformed with plasmid pET21a (+), an inducible expression vector (Novagen) in which the IL24 gene has been inserted between XhoI–BamHI sites. Host cells were transformed using calcium chloride and plated on LB agar plates containing 100 mg/L ampicillin.

Induction and expression of rhIL24 in culture flasks

Glycerol stocks were seeded in LB medium with ampicillin (1 g/mL), with shaking at 180 rpm at 37 °C overnight. Cultures were transferred into 30 mL LB medium and cultivated with rotation at 200 rpm at 37 °C. When the culture OD_{600} reached 0.8–1.0, lactose or IPTG were added separately to induce protein expression. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze rhIL24 expression levels.

3-L fermentation of rhIL24

The rhIL24 strain was removed from glycerol stock, seeded in LB medium, and rotated at 200 rpm at 37 °C. When the culture OD_{600} reached 0.8–1.0, 25 mL culture was transferred into 250 mL modified medium (10 g/L tryptone, 10 g/L yeast extract, 1.0 g/L potassium dihydrogen phosphate, 3.0 g/L potassium hydrogen phosphate, 4 g/L sodium chloride, 0.5 g/l glucose pH 7.2), and incubated with continuous shaking at 180 rpm at 37 °C for 8–10 h. The culture was transferred to 3 L fermenters containing fermentation medium (23 g/L trypton, 23 g/L yeast extract, 5 g/L sodium chloride, 1 g/L potassium dihydrogen phosphate, 3 g/L dipotassium hydrogen phosphate, 0.5 g/L ammonium chloride, and 2 g/L glucose). During cultivation, 20 % ammonia solution was used to maintain the pH at 6.9–7.0, and oxygen levels were maintained at 30 % by regulation of the oxygen feed rate, ventilation, and rotation speed. Lactose (6 g/L) was added to cell culture when cells reached mid-log phase. During induction, the pH was kept at 6.9–7.0 with oxygen level maintained above 30 % for 6 h, until final OD_{600} reached 9.0–10. Cells were harvested by centrifugation at 8,000 rpm for 30 min and stored at −20 °C [20].

Disruption, washing and isolation of inclusion bodies (IBs)

The harvested cell paste was dissolved in 50 mM Tris–HCl buffer (pH 8.0) containing 5 mM EDTA and 1 mM PMSF. Cells were lysed by homogenization (Constant Cell Disrupter, Daventry, UK) at 1.5 Kbar and IBs recovered by centrifugation at 8,000 rpm for 30 min at 4 °C. IBs were washed with 50 mM Tris–HCl buffer (pH 8.0) containing 5 mM EDTA. Finally, IBs were washed with distilled water to remove contaminating salt and detergent. The cell paste was centrifuged at 8,000 rpm for 30 min at 4 °C and the pellet used for estimating rhIL24 [16].

Two-step denaturing and one-step refolding of rhIL24 IBs

IBs were dissolved in 5 mL extraction buffer I (50 mM Tris–HCl, 50 mM NaCl, 10 mm beta-mercaptoethanol and 7 M guanidine-HCl, pH 8.0), and centrifuged at 12,000 rpm for 30 min at 4 °C. Supernatant was diluted in 200 mL dilution buffer (50 mM Tris–HCl, 1 mM EDTA, 50 mM NaCl, 10 mm beta-mercaptoethanol, pH 8.0). Precipitate was collected by centrifugation at 12,000 rpm for 30 min at 4 °C. The precipitate was further dissolved in 5 mL extraction buffer II (50 mM Tris–HCl, 50 mM NaCl, 10 mM beta-mercaptoethanol and 8 M Urea, pH 8.0). The solution was stirred for 20 min and centrifuged at 12,000 rpm for 30 min at 4 °C. Supernatant was collected and the total protein concentration measured by Bradford assay [3]. Protein concentration was adjusted to 8.0–10 mg/mL. The solution was added drop-wise up to 400 mL of refolding buffer (20 mm Tris, 1 mM EDTA, 1 mM GSH, 0.1 mM GSSG, pH 8.0) and stirred slowly with a magnetic stirrer at 4 °C overnight [23].

Diafiltration

Refolded rhIL24 was filtered with a 1.2 μm filter, concentrated, and buffer exchanged through a tangential flow filtration system (TFF) using nitrocellulose membrane cartridges with a 10 KD molecular weight cutoff, at a peristaltic pump flow rate of 3 mL/min. A volume of 20 mM sodium acetate buffer (pH 5.5) was pumped into the sample reservoir at 1 mL/min, and concentrated up to 25 % of

original volume. Conductivity was maintained at 2 mS/cm at pH 7.0. The sample yield was calculated by Bradford assay.

Cation exchange chromatography

Refolded protein pH was adjusted to 5–6 by adding 2 M acetic acid and loaded in a SP Sepharose column (AKTA Purifier, GE Healthcare, Piscataway, NJ, USA) at 20 °C and a flow rate of 3 mL/min. The column was equilibrated with three bed volumes of 20 mM sodium acetate buffer (pH 5.5). The refolded protein sample was loaded directly onto the column at the same flow rate and washed extensively with three bed volumes of the same buffer but with 0.5 M NaCl.

SDS-PAGE and western blot

SDS-PAGE (15 %) was performed according to the method of Laemmli [13]. Western blot analysis was carried out using anti-rhIL24 antibody (Abcam, Cambridge, MA, USA). Protein was transferred to nitrocellulose membrane and stained to verify effective transfer of samples. Membranes were blocked overnight at 4 °C with TBS (10 mM Tris–HCl, 150 mM NaCl, pH 7.5) containing 5 % non-fat milk. Two identical membranes were incubated for 4 h at 37 °C with rabbit anti-rhIL24 monoclonal antibody at 1:1,000, respectively. Membranes were washed three times with TBS-T (TBS with 0.05 % Tween 20) and incubated for 4 h at 37 °C with goat anti-rabbit IgG (HRP) antibody (Abcam) at 1:5,000. Immunoreactivity was visualized with diaminobenzidine (DAB) as substrate.

LC–MS/MS

RhIL24 peaks were collected following cation exchange chromatography, resuspended in menthol, and analyzed by UPLC-MS/MS. Analyses were carried out using an Ultra Performance Liquid Chromatography apparatus (UPLC, Intertek, Manchester, UK), equipped with a Waters Acquity PDA detector (Waters, Milford, MA, USA), and an Acquity UPLC BEH C18 column (100 × 2.1 mm, particle size 1.7 μm) (Waters). The mobile phase consisted of (A) water/acetonitrile, trifluoroacetic acid (TFA) (90; 10; 0.1 v/v); and (B) water, acetonitrile and TFA (10; 90; 0.1), with a gradient program of (B) 5–40 % for 0–10 min, (B) 40–100 % for 10–15 min and (B) 100–5 % for 15–20 min. The flow rate was 0.3 mL min^{-1}, and the column temperature was set to 30 °C. UV–vis absorption spectra were recorded online from 200–700 nm. Mass spectroscopic (MS) analysis of the collected fractions was performed using a SYNAPT Mass Spectrometer (Waters), operating in positive mode. The effluent was introduced into an electro spray source (source block temperature 100 °C, desolvation temperature 400 °C, capillary voltage 3.5 kV, and cone voltage 45 V). Argon was used as a collision gas (collision energy 16 eV) and nitrogen as a desolvation gas (500 L h^{-1}).

Bioactivity assay of rhIL24 in vitro

The growth promoting effect of rhIL24 on HepG2 cells was determined by standard MTT [3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide]) proliferation assay [15]. HepG2 cells were cultured in RPMI 1,640 medium with 10 % fetal bovine serum and incubated with 5 % CO_2 at 37 °C. Log phase HepG2 cells were diluted to 5.0 × 10^5/mL, and incubated for 24 h in 96-well plates with a serial dilution of rhIL24. After incubation, 20 μL MTT (5.0 mg/mL) was added to each well. Apoptosis-induced effects were measured on a plate spectrophotometer by reading the optical density (570 nm) of formazan, which is produced by viable cells from MTT. Survival rate was calculated according to Jianguo et al. [12].

$$\text{Survival rate}\,(\%) = (\mathrm{T} - \mathrm{B})/(\mathrm{U} - \mathrm{B}) \times 100\,\%$$

where T, treated: absorbance determined when tumor cells were exposed to rhIL24; U, untreated: absorbance of untreated cells; B, blank: absorbance when neither the drug nor MTT was added. The cytotoxicity was analyzed by GraphPad Prism5.

Results and discussion

Cloning and construction of the pET21a (+)-rhIL24

The hIL24 cDNA was inserted into pBV220. A positive clone (clone pBV220-rhIL24) was identified and isolated. The resulting pBV220-rhIL24 was digested with BamHI and XhoI, and ligated into vector pET21a (+) [26]. The pEt21a (+)-rhIL24 was verified by restriction enzyme analysis and direct sequencing. The resulting pEt21a (+)-rhIL24 was constructed without a 6XHis tag and EK sequence, allowing single-step purification by cation exchange chromatography with higher yield and quality.

Medium selection

Protein production in *E. coli* is increased significantly using a fermenter culture system. The most important factor influencing cell growth and protein production is the composition of the medium [21]. Addition of carbon and nitrogen sources into modified medium enhances enzyme production in *E. coli* [5]. Shake flask fermentation of rhIL24 was carried out with modified LB medium. Addition of yeast extract helps reduce acetic acid accumulation during

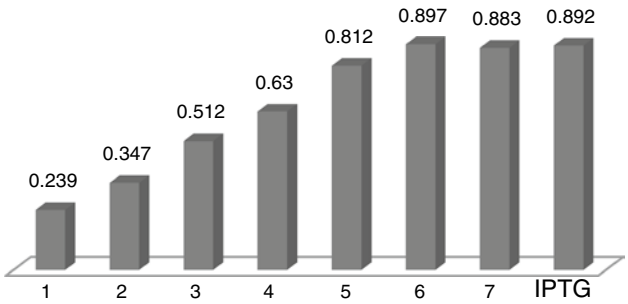
Table 1 Comparative quantification study of rhIL24 production in *E. coli*

	Yang et al. [23] reported recombinant human interleukin 24 production as fusion protein[a]	In current study recombinant human interleukin 24 production as non fusion protein
Source	*E. coli*, BEL21 (DE3)	*E. coli*, BEL21 (DE3)
Media	M9-CAA (15 L)	Modified medium. With addition of yeast extract and glucose (3 L)
Inducer	IPTG (0.5 mM)	Lactose (6 g/mL)
Harvested cell paste	24.6 g	115 g
Refolding	1. Affinity Chromatography (Fusion protein) 2. Gel filtration 3. Purified fusion protein added to refolding buffer 1 for 12 h 4. Refolding buffer II for 12 h 5. Ultra filtration	1. Direct dilution 2. Dialysis
Purification	1. Refolded fusion protein digestion by EK 2. SP fast flow column purification	1. SP column purification
Activity	IC_{50} for A549 cells 0.8 μg/mL (mol wt 18.3 KD)	IC_{50} for hepG2 cells 0.28 μM

[a] In the literature, rhIL24 expression was confirmed in viral or other expression systems and the production process was briefly described, except Yang et al. [23] described the entire bioprocess of rhIL24 in *E. coli*

limiting carbon conditions, and improves cellular yield of expressed protein. Yeast extract and glucose help maintain a higher growth rate during fermentation, while excreting little acetic acid [21]. We harvested 115 g of cell paste from 3 L fermenters by centrifugation at 8,000 rpm at 4 °C. This amount was much higher than that obtained from fermentation by Yang et al. [23]. The comparative quantification analysis of rhIL24 production in *E. coli* revealed the advantages for combination of ongoing biological applications for therapeutic production in current work (Table 1).

Table 2 Induction in 20 mL shake flask fermentation at 200 rpm



Lane # 1–7 lactose concentration (1–7 g/L). *Lane # 8* IPTG (ImM)

Lactose induction

Recombinant protein production is influenced by cellular growth rate and induction conditions [4]. Here, we used lactose as a substitution for IPTG for inducing rhIL24 expression in modified medium. Tian et al., originally described lactose as cheap and nontoxic substitute for IPTG for recombinant protein production in *E. coli*. Lactose induction increases the rate of bacterial protein productivity and shows no effect on biological activity of the target protein. In recombinant protein fermentation, lactose acted as a carbon source by *E. coli*-induced target protein expression at a low rate which is beneficial for soluble expression [20]. Lactose at a concentration of 1–7 g/mL or IPTG (1 mM) was used to produce rhIL24 by *E. coli* (Table 2). Although cells responded more slowly to lactose induction, a high rhIL24 expression was observed. Efficiency of lactose at a concentration of 6 g/mL equaled to 1 mM IPTG, as assessed by SDS-PAGE (Fig. 1a, b). IPTG toxicity and cost has resulted in it being removed as an inducer for large-scale production of recombinant human therapeutic proteins [11]. These results suggest that lactose should be preferred over IPTG for protein induction.

Cell lysis and inclusion body isolation

Cellular disruption was used to recover rhIL24 IBs from the *E. coli* cytoplasm. We applied both sonication and homogenization strategies to assess the efficiency of cell lysis. The cell lysis was tested at sonication pulses of 5, 10, 15, and 20 s and at different homogenizer pressures (600, 800, 1,200, and 500 bar).

The best recovery was obtained by passing the medium through a homogenizer two times at 2,500 bar and 4 °C. IB recovery was measured by the Bradford method. Homogenization to disrupt bacterial cells is an ideal strategy for commercial-scale production of recombinant human therapeutic proteins, and these were the conditions chosen to optimize. The optimization of early downstream processes,

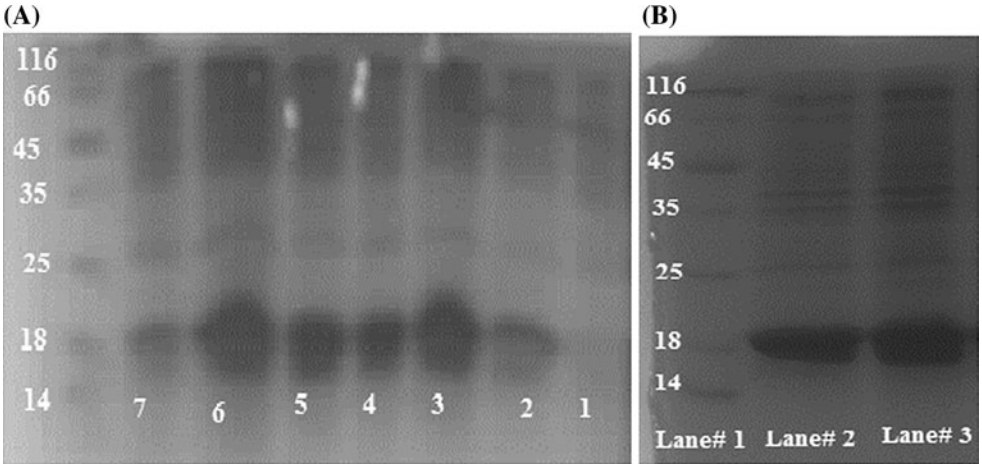


Fig. 1 SDS-PAGE analysis of lactose induction. **a** From right to left 1–7 g/L. Molecular weight markers. **b** Comparison of induction with IPTG or lactose. *Lane 1* molecular weight markers. *Lane 2* 1 mM IPTG. *Lane 3* 6 g/L IPTG

such as IB recovery, impacts the overall process yield and final product purity.

IB washes

IBs recovered by homogenization undergo several washes to remove endotoxins and host DNA. IB impurities interfere with refolding and significantly affect product yield and purity. IBs were treated with wash buffer and a final washing with ultra pure water, to remove detergents. The ultra pure water wash is time consuming but beneficial to limiting problems related to product contamination, and water is relatively inexpensive and a generally available resource for process scale-up (Fig. 2).

IB solubilization and refolding

The key step in IB solubilization is the development of an efficient and cost-effective denaturant. Ideally, a minimum amount of denaturant is needed to solubilize protein, allowing for full bioactivity recovery during refolding. At this stage, our process varied a little from conventional methods used in recombinant protein solubilization and refolding. Traditional approaches to solubilize IBs and refold rhIL24 had one denaturation step and one refolding step, with a common buffer composition. We used two denaturing steps and one refolding step (2DR). The first denaturing step was to dissolve IBs using extraction buffer I with 7 M guanidine, which precipitates guanidine-HCl denatured protein. The protein solution is then diluted with dilution buffer. The second denaturing step dissolves protein precipitate using extraction buffer II with 8 M urea. Refolding of rhIL24 was conducted by drop-wise dilution in refolding buffer. The principal advantage of the 2DR technique is for the two denaturing

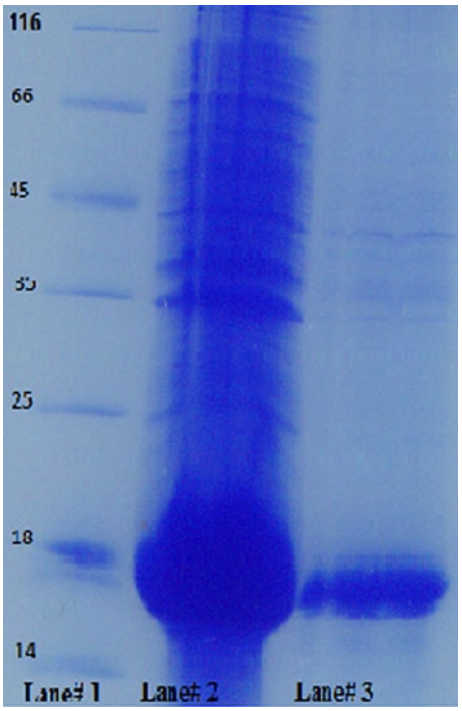


Fig. 2 SDS-PAGE analysis of rhIL24 inclusion bodies. *Lane 1* molecular weight markers. *Lane 2* rhIL24 fermented cell paste. *Lane 3* washed inclusion bodies

steps to completely unfold the misfolded protein in the IBs. Guanidine-HCl is then removed to obtain precipitated protein. The second denaturant, urea, is to dissolve protein precipitates. Another key aspect of 2DR is the precipitation of guanidine-HCl denatured protein through rapid dilution to minimize the protein loss in the supernatant. Guanidine-HCl denatures misfolded secondary structures into random coiled

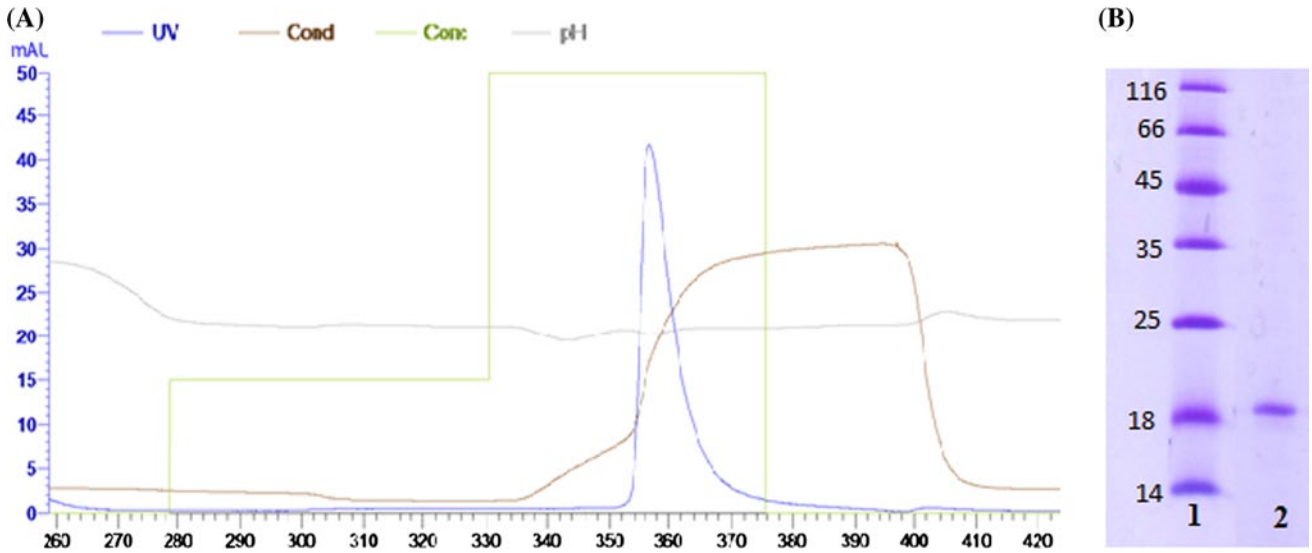


Fig. 3 SP Sepharose column purification of rhIL24. **a** Purification of rhIL24 by step gradient elution. **b** SDS-PAGE column purification of rhIL24. *Lane 1* molecular weight markers. *Lane 2* purified rhIL24

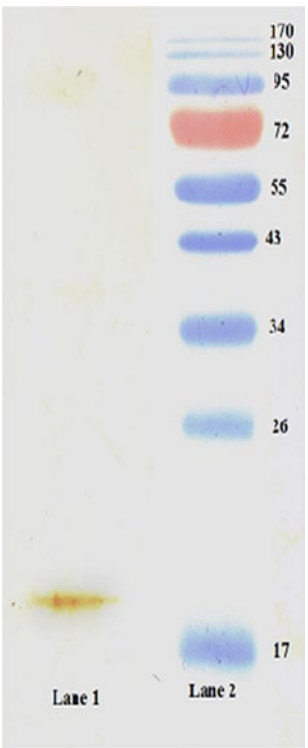


Fig. 4 Western blot analysis of rhIL24. *Lane 1* purified rhIL24. *Lane 2* molecular weight markers (SM0671)

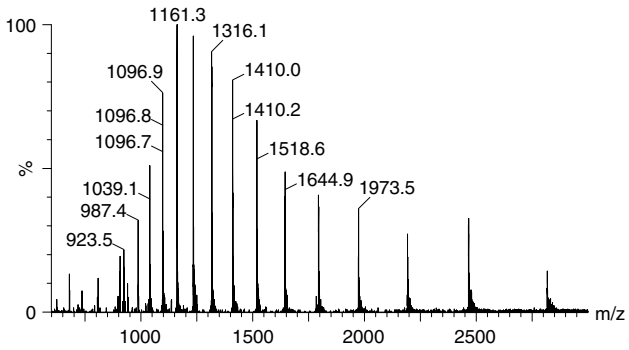


Fig. 5 rhIL24 mass-to-charge ratio (*m/z*) by TOF MS/MS+ at retention time 10.74 min

structures. Precipitated guanidine, once dissolved in urea, increases the yield of soluble protein. Protein refolding is performed by drop-wise addition of denatured IBs into refolding buffer, with rapid stirring [24]. During the protein folding process, the formation of correct structures is obtained from homogeneous but partially folded intermediates. During the refolding process, removal of denaturant allows the protein to fold properly. At the same time, sufficient denaturant should be left in the refolding buffer to allow the protein to fold/refold several times and attain the proper conformation. The 2DR method is thus more efficient than the traditional one-step denaturing and refolding process. The 2DR method is simple and generally applicable for obtaining active recombinant protein for basic research and industrial purposes.

Cation exchange chromatography

The final downstream processing step is used to purify properly folded protein by removing endotoxins, nucleic acids, and protein related impurities. The AKTA Purifier with a SP Sepharose column was used in a single-step purification process. The results indicated effective removal of

impurities and recovery of a monomer peak (Fig. 3a). The presence and purity of the desired rhIL24 protein was confirmed by SDS-PAGE, which indicated a purity of ~98 %, with a single band of 19.7 KD (Fig. 3b).

Western blot analysis and LC–MS/MS

Purified rhIL24 sample was transferred from SDS-PAGE to nitrocellulose membrane and immunodetected with anti-IL24 serum, revealing a typical profile (Fig. 4). Western blot analysis confirmed rhIL24 expression in samples obtained by single-step purification, and was further analyzed by LC–MS/MS. LC–MS/MS provides retention time and the molecular weight. From the total ion current, a retention time of 10.74 min was selected and the m/z interpreted as the mass value of rhIL24. This process was used to assign a mass value of 19.72 KD to rhIL24, as identified through MS/MS spectra (Figs. 5, 6). LC–MS/MS is a simple, low-cost, but high-sensitivity analysis method used in proteomic research to identify and provide quantitative protein information [9].

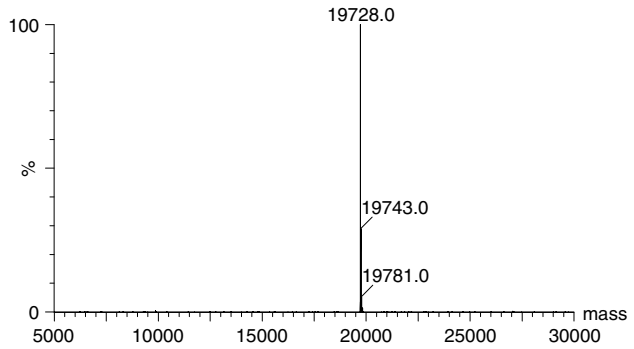


Fig. 6 Molecular weight determination of rhIL24 by LC–MS/MS. Based on *m/z* of rhIL24, the predicted mass was 19.72 KD

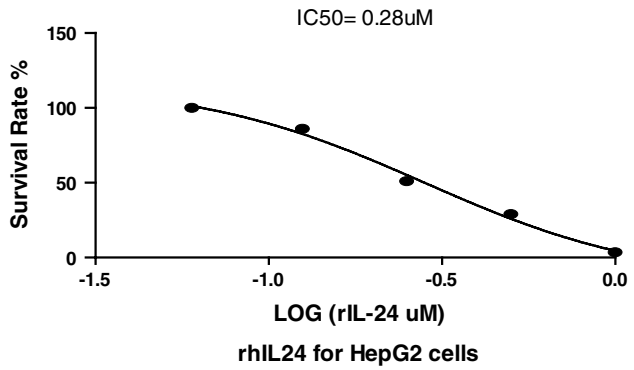


Fig. 7 Effect of rhIL24 on HepG2. The cells were treated with the indicated dilutions of rhIL24 in 96-well plates. Cell growth was evaluated by MTT assay, and absorbance at 570 and 630 nm measured

Biological activity assay of rhIL24

The biological activity of rhIL24 was tested in a standard protocol of cell proliferation assay using HepG2 hepatocellular carcinoma cells and L02 normal liver cells [15]. RhIL24 stimulates HepG2 proliferation in a dose-dependent manner. Specific activity of the rhIL24 was calculated by regression analysis, revealing an IC_{50} for HepG2 cells of 0.28 μM (Figs. 7, 8). This demonstrates that the purified rhIL24 possesses elevated biological activity against HCC, with no effect on normal liver cells. RhIL24 possesses good activity with no significant harmful effects on normal human epithelia or fibroblasts [14, 17]. However, it has been noted that the dose and duration of action affects MDA-7 apoptosis [19]. Xue et al., reported Ad.MDA-7 as a growth inhibitor of apoptosis in HCC cells, and that Hep3B had no effect on the normal liver cell line L02. Anti-apoptosis protein (Bcl-2) expression decreased in Ad.MDA-7-treated HCC cells but remained unchanged in liver normal cells [22].

Conclusions

Cost effective production of recombinant human interleukin 24 correlated with expression host, selection of

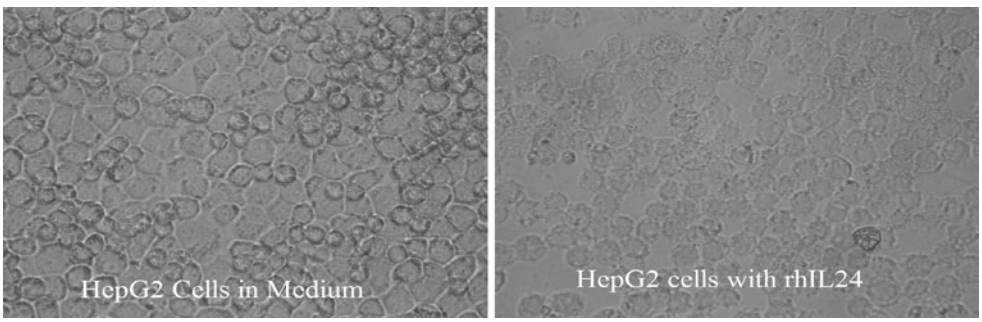


Fig. 8 HepG2 cells cultured in growth medium. Cells were treated with different dilutions of rhIL24 after 24 h. HepG2 cells at higher rhIL24 concentrations displayed low level cell viability

fermentation media and route of production. *E. coli* is a cheap and ideal host for large-scale production of therapeutic protein because of high productivity when compared to other expression systems. Therefore, we used *E. coli* strain BL21 (DL3) (Novagen, Darmstadt, Germany). Lactose as a cheap and nontoxic inducer increased bacterial productivity without any effect on biological activities replaced IPTG beneficially. Meanwhile, the selection of fermentation media for large-scale production of therapeutic rhIL24 is a key factor to reduce the production cost. The glucose and yeast extract combination in fermentation media not only increased the biomass but also provided a cheap carbon source for fermentation. The bottle neck of therapeutic protein production in *E. coli* is inclusion bodies refolding. Traditional procedure for refolding resulted in a sharp decrease in the final yield. The two-step denaturing and one-step refolding process made inclusion bodies refolding easier and minimized the loss of therapeutic protein. The single step purification made the process simple resulted in the production of rhIL24 with high purity. The combination of ongoing bioprocessing technological advances will increasingly enable manufacturing of rhIL24 quicker, simpler and at significantly reduced cost.

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References

1. Amirzada MI, Jin J (2012) Therapeutic applications of interleukin 24, a review. Trop J Pharm Res 12(6):1023–1027
2. NIH Report (2002) Assessment of Adenoviral vector safety and toxicity: report of the National Institute of Health Recombinant DNA Advisory committee. Human gene therapy. Marry Ann Liebert, Inc. 13, pp 3–13. http://oba.od.nih.gov/oba/rac/adsat_rpt.pdf. Accessed 15 Mar 2013
3. Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal Biochem 72:248–254
4. Chen SJ, Chang MC, Cheng CY (1997) Effect of induction conditions on production and excretion of Aeromonas hydrophilia chitinase by recombinant *Escherichia coli*. J. Ferment Bioeng 84(6):610–613
5. Chen Y, Xing XH, Ye F, Kuang Y, Luo M (2007) Production of MBP-hepA fusion protein in recombinant *Eschericia coli* by optimization of culture medium. Biochem Eng J 34:114–121
6. Corchero JL et al (2012) Unconventional microbial systems for the cost-efficient production of high-quality protein therapeutics. Biotechnol Adv. doi:10.1016/J.Biotechadv.2012.09.001
7. Cosat FP, Oliveira AD, Meirelles R, Machado M, Zanesco T, Surjan R, Chammas MC, SouzaRocha MD, Morgan D, Cantor A, Zimmerman J, Kuster N, Barbaults A, Pasche B (2011) Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic field. Br J Cancer 105:640–648
8. Dehaghani SA, Babaeipour V, Mofid, Divsalar A, Faraji F (2010) An efficient purification method for high recovery of recombinant human granulocyte colony stimulating factor from recombinant E. coli. Int J Environ Sci Dev 1(2):111–114
9. Faca V, Coram M, Phanstiel D, Glukhova V, Zhan Q, Fitzgibbon M, Mclntosh M, Hanash S (2006) Quantitative analysis of acrylamide labeled serum protein by LC–MS/MS. J Proteome Res 5:2009–2018
10. Fuson KL, Zheng M, Craxton M, Pataer A, Ramesh R, Chada S (2009) Structural mapping of post-translational modification in human interleukin-24. Role of n-linked glycosylation and disulfide bonds in secretion and activity. J Biol Chem 284(44):30526–30533
11. Jana S, Deb JK (2005) Strategies for efficient production of heterologous protein in *Escherichia coli*. Appl Microbial Biotechnol 67:289–298
12. Jianguo C, Xiaoqing T, Hong Z, Bo P (2004) Reversal of multidrug resistance by Neferine in Adriamycin resistant human breast cancer cell line MCF-7/ADM. The Chinese German. J Clin Oncol 3(2):93–96
13. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
14. Lebedeva IV, Sauane M, Gopalkrishnan RV, Sarkar D, Su ZZ, Gupta P et al (2005) mda-7/IL-24: exploiting cancer's Achilles' heel. Mol Ther 11:4–18
15. Mosmann T (1983) Rapid colorimetric assay for the cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
16. Patra AK, Mukhopadhyay R, Mukhija R, Krishnan A, Garg LC, Panda AK (2000) Optimization of inclusion bodies solubilization and renaturation of recombinant human growth hormone from *Escherichia coli*. Protein Expr Purif 18:182–192
17. Qian W, Liu J, Tong Y, Yan S, Yang C, Yang M et al (2008) Enhanced antitumor activity by a selective conditionally replicating adenovirus combining with MDA-7/interleukin-24 for B-lymphoblastic leukemia via induction of apoptosis. Leukemia 22:361–369
18. Sauane M, Gopalkrishnan RV, Choo HT, Gupta P, Lebedeva IV, Yacoub A, Dent AP, Fisher PB (2004) Mechanistic aspects of mda-7/Il-24 cancer cell selectivity analysed via a bacterial fusion protein. Oncogene 23:7679–7690
19. Shanker M, Gopalan B, Patel S, Bocangel D, Chada S, Ramesh R (2007) Vitamin E succinate in combination with mda-7 results in enhanced human ovarian tumor cell killing through modulation of extrinsic and intrinsic apoptotic pathways. Cancer Lett 254:217–226
20. Tian H, Tang L, Wang Y, Wang X, Guan L, Zhang J, Wu X, Li X (2011) Lactose induction increases production of recombinant keratinocyte growth factor-2 in *Escherichia coli*. Int J Pept Res Ther 17:123–129
21. Tripathi NK, Sathyaseelan K, Jana AM, Rao PVL (2009) High yield production of heterologous proteins with *Escherichia coli*. Def Sci J 59(2):137–146
22. Xue XB, Chen K, Wang CJ, Zheng JW, Yu Y, Peng ZH, Wu ZD (2008) Adenovirus vector expressing mda-7 selectively kills hepatocellular carcinoma cell line Hep3B. Hepatobiliary Pancreat Dis Int 7:509–514
23. Yang J et al (2007) Expression, purification and characterization of recombinant human interleukin 24 in *Escherichia coli*. Protein Expr Purif 53:339–345
24. Yang Z, Zhang L, Zhang Y, Zhang T, Feng Y, Lu X, Lan W, Wang J, Wu H, Cao C, Wang X (2011) Highly efficient production of soluble protein from inclusion bodies by a two step denaturing and refolding method. PLoS One 6(7):e22981
25. Zhang X, Kang X, Shi L, Li J, Xu W, Qian H, Wu M, Yin Z (2008) MDA-7/IL-24 induces apoptosis in human HepG2 hepatoma cells by endoplasmic reticulum stress. Oncol Rep 20:437–442
26. Zhen Min Y, Ye-Han Z, Wei-Hua S, Jing-Cheng M, Shao-Hua W, Kang W, Ji-Cheng Y (2005) The expression of recombinant protein of human interleukin 24 and its anti-tumor mechanism. Ch J Biotech 21(5):719–724