Journal of Medical Hypotheses and Ideas (2014) 8, 7–13

Available online at www.sciencedirect.com

Journal of Medical Hypotheses and Ideas

journal homepage: www.elsevier.com/locate/jmhi

REGULAR ARTICLE

Overexpression of MDA-7/IL-24 as an anticancer cytokine in gene therapy of thyroid carcinoma

Mehri Hajikhan Mirzaei [a], Abdolreza Esmaeilzadeh [b,c,*]

[a] Department of Medical Genomics, Graduate School of Frontier Science, University of Tokyo, Tokyo, Japan
[b] Department of Immunology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[c] Cancer Gene Therapy Research Center, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

Received 31 January 2013; revised 2 May 2013; accepted 14 June 2013
Available online 1 July 2013

KEYWORDS
Thyroid carcinoma;
MDA-7/IL-24;
Xenograft mouse model;
HTori cell;
Immune gene therapy

Abstract The annual incidence of thyroid cancer worldwide is alarming. Despite current various treatments such as surgical resection, radioiodine therapy and chemotherapy/radiotherapy, thyroid carcinoma remains a lethal cancer. Assuredly, the operative and new treatment strategies are necessary to control this malignancy. Gene therapy is regarded as one of the most reliable novel therapeutic methods for hopeless cases of thyroid cancer and those who do not respond to the prevalent treatments. Accumulated evidence suggests that interleukin-24 (IL-24), also known as melanoma differentiation-associated gene-7, has very important roles in regulation of cell differentiation, cell growth and apoptosis, and it is also a promising anticancer agent. Here, we propose that it could be advantageous to evaluate the anti-tumoural effect of IL-24 in a mouse xenograft model of thyroid cancer.

Introduction

The thyroid is a main human endocrine gland that controls heart rate, blood pressure, body temperature and metabolism [1]. Thyroid carcinoma is the most frequent endocrine malignancy [2], and its incidence is more common than ovarian, urinary bladder or pancreatic cancer, with an incidence 3 times higher in women than in men [3].

Abbreviations: mda, melanoma differentiation-associated; IL-24, interleukin 24; PTC, papillary thyroid cancer; ELISA, enzyme-linked immunosorbent assay; IL-20R, interleukin 20 receptor; TGF-β, transforming growth factor β; (GADD), growth arrest and DNA damage; JAK/STAT, Janus-activated kinase/signal transducers and activators of transcription.

* Corresponding author at: Department of Immunology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran. Tel.: +98 241 4240301 299, mobile: +98 912 1414281; fax: +98 241 4249553.
E-mail address: a46reza@zums.ac.ir (A. Esmaeilzadeh).

2251-7294
URL: www.tums.ac.ir/english/
doi:http://dx.doi.org/10.1016/j.jmhi.2013.06.002

Patients with radioactive iodine-refractory and metastatic thyroid cancer have few therapeutic options, with a 30% response rate to systemic chemotherapy and no proven survival benefit [4].

Papillary thyroid cancer (PTC) is the most common histological type of thyroid malignancy [5]. The genetic malfunctions that cause thyroid neoplasia include rearrangements and point mutations in some genes, such as *RAS*, *RET* and *p53*. Specially, deficiency in DNA repair, apoptosis and tumour suppressor gene malfunction result in thyroid tumours [5].

Despite many traditional treatments for thyroid cancer, including surgical resection, radioiodine therapy, thyrotropin (thyroid stimulating hormone, TSH)-suppressive thyroxine treatment and chemotherapy/radiotherapy, the chance of survival in patients is low. Thus, alternative approaches, such as gene therapy, are of interest, and fortunately, thyroid tumours are a good target, for two reasons. First, certain gene promoters expressed in the thyroid tumours have no or very limited expression elsewhere, and second, if the therapy leads to destruction of all normal thyroid tissue, this would be inconsequential to destruction of the other tissues [5,6]. Gene therapy can be effective and safe, but for each approach there is an evaluation between the degree of effectiveness and undesirable toxic effects that may occur due to non-selectivity. For example, Nishihara et al. have reported the effect of Re-TG-tk/Cre-loxP on FRTL-5, FRTC and FRO cell lines, which has extrathyroidal tissue toxicity. In addition, Zang et al. had shown an anti-tumoural effect of Ad–TG-tk and Ad–CMV-tk in FRTL-5, HEPG2, COS-1, MTC, HeLa and GH3 cells which were not effective in the anaplastic form besides severe liver damage. Barzon et al. have evaluated Re-tk-IL-2 in human ATC cell line. This strategy had low efficiency in addition to the mild side effects. Adenoviruses and other viruses have been engineered for selective replication within neoplastic cells. It is almost impossible to deliver the virus to all the tumoural cells; therefore, the uninfected tumoural cells will continue to grow [7].

In this hypothesis, we focussed on the tumour suppressive function of melanoma differentiation associated gene-7/interleukin-24 (MDA-7/IL-24) in a xenograft mouse model for thyroid carcinoma. Based on the sequence homology, FISP is the mouse orthologue of mda-7/IL-24 [8]. MDA-7, also known as IL-24, is a member of the class II/IL-10 cytokine family [9,10] and has been mapped to 1q32.2-q41 [11,12]. MDA-7 is expressed by the spleen, thymus and immune cells, including TH2 cells, B cells, natural killer (NK) cells, dendritic cells, monocytes and melanocytes [13–15]. Further, MDA-7 is expressed in the villi, decidual tissue, villous column, trophoblasts, stroma and blood vessels [16]. The IL-20R1:IL-20R2 complex is a heterodimer receptor for IL-24. The latter also signals through an IL-22R1/IL-20R2 heterodimer (type II complex) [10,17–23]. Generally, epithelial cells are major expression sites for IL-24 receptors [24]. A high level of IL-20R1 messenger RNA (mRNA) expression was indicated in the skin, testis, heart, placenta, salivary gland and prostate. Mild expression was detected in brain, lung, stomach, pancreas, ovary, uterus, thyroid and adrenal glands. Scarce expression was observed in the kidney, liver, colon, muscle and small intestine [25]. Mda-7 has at least two separate functions. It mostly acts as a cytokine at low concentration. However, overexpression of IL-24 at the supra-physiological level shows an irreversible cancer cell growth inhibitory function and G2/M cell cycle arrest, reversal of the malignant phenotype and terminal differentiation, without negative effects on normal cells [26,27]. When IL-24 as a cytokine binds to its receptor, Janus activating kinase 1 (JAK1) is activated and causes the receptor phosphorylation and creates binding sites for signal transducers and activators of transcription 1 (STAT1) and STAT3, which are also phosphorylated by JAK1. The phosphorylated STATs, in the nucleus, promote the transcription of cytokine-regulated genes. This is the first function of mda-7 [28,29]. However, it does not depend on this pathway to induce apoptosis [24]. Recombinant (r)IL-24 stimulates genes of the extrinsic and intrinsic apoptotic pathway such as Bax, Bak, Bid, Casp8, cytochrome *c* oxidase subunit VIc (COX6C) and cytochrome *c* oxidase subunit VIIb (COX7B) [30]. In fact, IL-24-induced apoptosis is conducted through down-regulation of Bcl-2, Bax and Akt [13]. However, Mda-7 induces the secretion of interferon-beta (IFN-beta), which subsequently leads to interferon regulatory factor (IRF-1) regulation and Fas/tumour necrosis factor-related apoptosis-inducing ligand (Fas/TRAIL) activation. Furthermore, MDA-7 has been implicated in inducing cell death with the contribution of ceramide, a promoter of apoptosis and a key mediator of the endoplasmic reticulum (ER) stress pathway [31]. Mda-7/IL-24 binding to BiP/GRP78 inactivates this ER-chaperone protein by increased protein kinase RNA-activated (PKR)-like endoplasmic reticulum kinase (PERK) autophosphorylation and increased phosphorylation of the downstream PERK target (eukaryotic initiation factor 2, eIF2) [32,33] and therefore could lead to a general suppression of protein expression, particularly of anti-apoptotic proteins, such as Bcl2, Bcl-XL, induced myeloid leukaemia cell differentiation protein (MCL-1) and cellular FLICE-like inhibitory protein (c-FLIP) [31] Mda-7/IL-24 induced ER stress and ceramide accumulation, which triggered autophagy.

Producers of reactive oxygen species (ROS) enhance MDA-7/IL-24 lethality. Overexpression of MDA-7/IL-24 in renal cell carcinoma caused plasma membrane clustering of CD95 and CD95 association with pro-caspase 8, which correlated with enhanced cell killing [34]. IL-24 also has immune stimulatory activity. It activates IL-6, tumour necrosis factor-α (TNF-α) and interferon production and has been shown to significantly down-regulate transforming growth factor-β (TGF-β) [35–37].

The hypothesis

Thyroid cancer is the sixth most common type of cancer among women and it is increasing more rapidly than any other cancer. Therefore, development and evaluation of novel treatment strategies, including immune gene therapy, are urgently needed. In this proposal, we suggest inducing IL-24 gene expression in the HTori human thyroid cell line and injection of this cell line into a xenograft system. In the last years, IL-24 has been introduced as a novel tumour suppressor gene

and apoptosis enhancer in many types of cancer cell lines and is even applied in patients with some types of malignancies. We expect that IL-24 can enhance malignant cell death through apoptosis and other possible pathways in a thyroid mouse model without any effect in normal cells.

Evaluation of hypothesis

- *Cell culture:* The human thyroid epithelial cell line (HTori-3) is grown in phenol red-free RPMI 1640, supplemented with 1% (v/v) antibiotics/antimycotics, 2 mmol/L of L-glutamine and 10% (v/v) foetal calf serum (FCS) [38].
- *Construction of recombinant adenovirus:* The IL-24 expression cassette is cloned into the adenovirus shuttle plasmid pCA13 to form pCA13–IL-24 and cut with *Bgl*II to clone into ZD55 to form pZD55–IL-24. Replication-defective adenovirus Ad–IL-24 is generated in HTori cells by homologous recombination. Recombinant adenovirus is amplified in HTori cells and purified by caesium chloride-gradient ultracentrifugation [34].

Briefly, recombinant adenovirus plasmid pAd–mda-7 carrying human mda-7/IL-24 complementary DNA (cDNA) is transfected into the human thyroid epithelial cell line (HTori-3) by Lipofectamine 2000 reagent, leading to the formation of the recombinant adenovirus, Ad–IL-24. At the same time, the recombinant adenovirus Ad-GFP carrying green fluorescent protein (GFP) is constructed as a control.

- *Tumour xenograft in nude mice:* A total of 40 athymic mice (AthymicNCr-nu/nu; 4 weeks old) are prepared. The first group ($n = 10$) contains control animals, which do not receive any injection. The remaining mice are divided into three categories of 10 animals each, including the following:
  (a) Ten mice are selected for HTori cell-line inoculation. HTori cells (5×10^6) in 200 µl of suspension mixture are injected subcutaneously (s.c.) into the right flank of athymic mice. Tumours are measured weekly and the 'end' point was reached when the tumours reached 3 cm in diameter [38].
  (b) The second group received HTori cells and empty adenovirus vector.
  (c) The next group includes mice that are injected with HTori cells according to the above instruction. This group received Ad–IL-24 (Fig. 1).
- RNA isolation and reverse transcriptase-polymerase chain reaction (RT-PCR): RNA from cells is extracted using an RNA extraction kit and cDNA synthesis and RT-PCR are performed.
- MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay: The cytotoxic activity of Ad–IL-24 is determined based on cytotoxicity to HTori cells, using the MTT assay.
- Enzyme-linked Immunosorbent Assay (ELISA): ELISA is performed to measure level of IL-24 in blood.
- Terminal deoxynucleotidyl transferase deoxy-uridinetriphosphate (dUTP) nick end labelling (*TUNEL*) test: This test is used to assay DNA fragmentation, as a marker of apoptosis [39,40].
- Evaluating IFN-gamma, Granzyme B, TNF-alpha and IL-24 through ELISA assay.

Discussion and conclusion

Given the lack of a promising cure for thyroid malignancy, cytokine gene therapy for cancer has been recognised as an efficient and novel treatment with the minimum side effects [5,6]. Among cytokines, mda-7/IL-24 as a new tumour suppressor gene has been investigated in animal models and *in vitro* studies [26,27]. Many studies show cytotoxic effects of IL-24 in various types of cancer cell lines, including glioma, ovarian, breast, lung, liver, pancreatic, gastric, colorectal, renal and prostate cancer cells [41,42]. In addition, overexpression of mda-7 in cervical cancer, fibrosarcoma, melanoma, hepatoma and osteosarcoma cell lines led to programmed cell death. Furthermore, the anti-tumour activity of IL-24 has been established in some tumour xenograft models and even in patients with advanced solid cancers [13,14,28,43]. MDA-7 also influences endothelial cells and has an anti-angiogenic effect within the tumour vasculature [13,9]. A Phase I/II clinical trial in patients with advanced carcinomas involving intratumoural administration of mda-7/IL-24 has documented that this gene is safe and well tolerated by patients and a single virus injection elicits apoptosis in a majority of the tumours [44,45].

cDNA libraries produced from H0-1 cells treated with IFN-beta + mezerein (MEZ) led to identification of this gene [46,47].

Our hypothesis suggested that Ad–IL-24 can inhibit the growth and proliferation of human epithelial thyroid HTori cells in tumour-bearing mice. IL-24 is a regulator of cell differentiation, cell growth and apoptosis and a promising anticancer agent in many types of tumour cell lines without toxic effects in 'normal' cell types [40,48]. This cytokine can induce both intrinsic and extrinsic apoptosis pathways. In the extrinsic pathway, IL-24 efficiently could suppress tumours through TNF-α and activation of the caspase 8–caspase 3 [48]; in the intrinsic pathway, induction of caspase 9 and *bax* gene expression were observed [37]. In ovarian cancer, mda-7/IL24 was reported to induce the extrinsic apoptosis pathway and also kill multiple renal carcinoma cell lines, also via activation of the CD95/FAS receptor [32]. IL-24-induced inhibition of cell proliferation was associated with the transcriptional up-regulation of the cell cycle inhibitors mediated by STAT3 activation [49,50]. Another study demonstrated the potential anti-tumour activity of IFN-α combined with IL-24 in hepatocellular carcinoma (HCC) both *in vitro* and *in vivo* [51]. Interestingly, studies have shown that one mechanism of clinically effective IL-2 therapy may be the direct action of IL-2 on up-regulation of the tumour suppressor IL-24 [52]. Mda-7/IL-24 also can enhance tumour sensitivity to radiation therapy [53,54]. Recent studies have demonstrated that IL-24 can regulate ER stress following the binding of the protein to BiP/GRP78. This binding eventually leads to the phosphorylation of eIF2 and therefore to a general suppression of anti-apoptotic proteins, such as Bcl-XL, MCL-1 and c-FLIP [31]. Further *in vivo* data showed that IL-24 suppressed tumour growth through up-regulating the expression of bax and down-regulating the

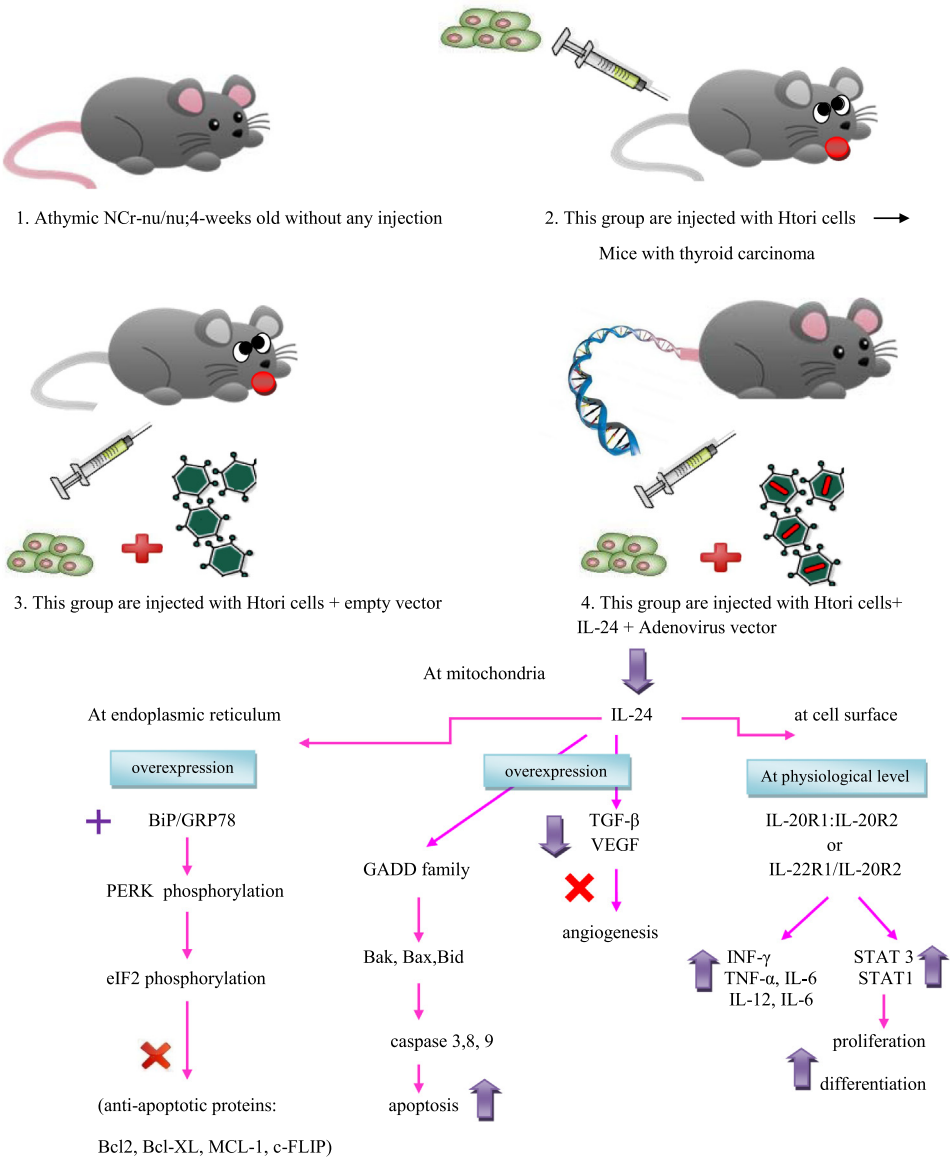

Figure 1 IL-24 as an anticancer cytokine in gene therapy of thyroid carcinoma schematic design of hypothesis procedure. Function of IL-24 in different cells.

expression of bcl-2 and vascular endothelial growth factor (VEGF) [28,29,49,55,56].

Moreover, IL-24 induces cancer cell-specific oxidative stress by generation of ROS followed by mitochondrial dysfunction uniquely in cancer cells [43]. Therefore, the canonical mechanism by which diverse cytokines, including members of the IL-10 gene family, are proposed to mediate bioactivity is by binding to defined cell surface receptors and activating JAK/STAT signalling pathways. In the case of mda-7/IL-24, JAK1/STAT3 activation is induced after binding of the Mda-7/IL-24 protein to the IL-20R1/IL-20R2 and IL-22R1/IL-20R2 receptors. Meanwhile, cell killing by mda-7/IL-24 did not require functional cell-surface receptors or STAT activation, and inhibition of tyrosine kinase activity or infection of cancer cells defective in specific components of the JAK/STAT signalling pathway still elicited apoptosis [37,55]. However, Mda-7 expression is substantially reduced in malignant breast tissue and low transcript levels are significantly associated with unfavourable pathological parameters. Mda-7 probably offers utility as a prognostic marker in thyroid carcinoma [13].

In conclusion, cytokine gene therapy demonstrates safety and effectiveness in thyroid carcinoma. Specially, IL-24 could act as an effective anticancer agent in this cancer via inducing apoptosis, according to recent documents on many other types of cancer.

Overview Box

First Question: What do we already know about the subject?

Thyroid cancer incidence is increasing more rapidly than any other malignancy. The expected annual number of newly diagnosed thyroid cancer cases in the US has reached 37,340. Despite current treatments for thyroid cancer, other efficient approaches are necessary. It is verified that IL-24 has tumour suppressor function through inducing both intrinsic and extrinsic apoptosis pathways. Interestingly, similar effects are not apparent following transduction into their non-malignant counterparts. IL-24 can suppress cancer cell growth, induce cancer cell apoptosis, inhibit angiogenesis and enhance the anti-tumour activity of radiotherapy, chemotherapy and targeting gene-virotherapy.

Second Question: What does your proposed theory add to the current knowledge available, and what benefits does it have?

IL-24 could be considered a novel candidate in the gene therapy of thyroid cancer. It could suppress the thyroid tumoural cell growth and angiogenesis conspicuously through up-regulating the expression of pro-apoptotic proteins (bax and Bak) and down-regulating the expression of bcl-2, Bcl-xL and VEGF, promoting a shift from survival to programmed cell death.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?

To test this idea, initially epithelial thyroid tumoural cells are induced in a mouse model via an adenovirus vector. Then, the anti-tumoural effect of IL-24 could be assessed by methods of measurement of biomarkers in blood levels.

In last phase, a clinical trial could be performed.

Acknowledgement

I am very thankful to my supervisor Dr Esmaeilzadeh who inspired and encouraged me for this hypothesis.

References

[1] Al-Humadi H, Zarros A, Al-Saigh R, Liapi Ch. Genetic basis and gene therapy trials for thyroid cancer. Cancer Genom Proteom 2010;7:31–50.

[2] Thosani S, Hu MI, Ahn P, Lamont JP. Thyroid and parathyroid cancers. 14th ed. Cancer Management; 2011.

[3] Wartofsky L. Increasing world incidence of thyroid cancer: increased detection or higher radiation exposure? Hormones 2010;9:103–8.

[4] Perez CA, Arango BA, Velez M, Raez LE, Santos ES. Emerging role of multikinase inhibitors for refractory thyroid cancer. Biol Targets Ther 2012;6:257–65.

[5] Li X, Abdel-Mageed AB, Kandil E. BRAF mutation in papillary thyroid carcinoma. Int J Clin Exp Med 2012;5:310–5.

[6] Spitzweg C, Morris JC. Gene therapy for thyroid cancer: current status and future prospects. Thyroid 2004;14:424–34.

[7] Xian J, Yang H, Lin Y, Liu S. Combination nonviral murine interleukin 2 and interleukin 12 gene therapy and radiotherapy for head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2005;131(12):1079–85.

[8] Kunz S, Wolk K, Witte E, Witte K, Doecke WD, Volk HD, et al. Interleukin (IL)-19, IL-20 and IL-24 are produced by and act on keratinocytes and are distinct from classical ILs. Exp Dermatol 2006;15:991–1004.

[9] Wang M, Tan Z, Zhang R, Kotenko SV, Liang P. Interleukin 24 (MDA-7/MOB-5) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. J Biol Chem 2002;277:7341–7 [Epub 2001 Nov 12].

[10] Imaeda H, Nishida A, Inatomi O, Fujiyama Y, Andoh A. Expression of interleukin-24 and its receptor in human pancreatic myofibroblasts. Int J Mol Med 2011;28(6):993–9. http://dx.doi.org/10.3892/ijmm.2011.793.

[11] Blumberg H, Conklin C, Xu WF, Grossmann A, Brender T, Carollo S, et al. Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell 2001;104:9–19.

[12] Witte E, Witte K, Warszawska K, Sabat R, Wolk K. Interleukin-22: a cytokine produced by TNK and NKT cell subsets, with importance in the innate immune defense and tissue protection. Cytokine Growth 2010:365–9.

[13] Patani N, Douglas-Jones A, Mansel R, Jiang W, Mokbel K. Tumour suppressor function of MDA-7/IL-24 in human breast cancer. Cancer Cell Int 2010;10:29.

[14] Sainz-Perez A, Gary-Gouy H, Gaudin F, Maarof G, Marfaing-Koka A, de Revel T, et al. IL-24 induces apoptosis of chronic lymphocytic leukemia B cells engaged into the cell cycle through dephosphorylation of STAT3 and stabilization of p53 expression. J Immunol 2008;181:6051–60.

[15] Sarkar D, Su ZZ, Lebedeva IV, Sauane M, Gopalkrishnan RV, Dent P, et al. Mda-7 (IL-24): signaling and Functional Roles. BioTechniques 2002;33:S30–9.

[16] Cheng H, Zou L. IL-24 expression at maternal-fetal interface and its roles in trophoblast invasion. J Huazhong Univ Sci Technol Med Sci 2008;28(4):456–9. http://dx.doi.org/10.1007/s11596-008-0418-9.

[17] He M, Liang P. IL-24 transgenic mice: in vivo evidence of overlapping functions for IL-20, IL-22, and IL-24 in the epidermis. J Immunol 2010;184:17931798. http://dx.doi.org/10.4049/jimmunol.0901829.

[18] Chan JR, Blumenschein W, Murphy E, Diveu C, Wiekowski M, Abbondanzo S, et al. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2–dependent mechanisms with implications for psoriasis pathogenesis. J Exp Med 2006;203:2577–87.

[19] Zhu H, Yang ZB. Expression pattern of mda-7/IL-24 receptors in liver cancer cell lines. Hepatobiliary Pancreat Dis Int 2009;8:402–6.

[20] Su L, Liao Q, Wu Y, Chen X. Kaposi's sarcoma-associated herpesvirus-encoded LANA down-regulates IL-22R1 expression through a cis-acting element within the promoter region. PLoS One 2011;6:e19106. http://dx.doi.org/10.1371/journal.pone.0019106.

[21] Logsdon NJ, Deshpande A, Harris BD, Rajashankar KR, Walter MR. Structural basis for receptor sharing and activation by interleukin-20 receptor-2 (IL-20R2) binding cytokines. Proc Natl Acad Sci USA 2012;109:12704–9. http://dx.doi.org/10.1073/pnas.1117551109 [[Epub 2012 Jul 16]].

[22] Coondoo A. Cytokines in dermatology – a basic overview. Indian J Dermatol 2011;56:368–74.

[23] Kotenko SV. The family of IL-10-related cytokines and their receptors: related, but to what extent? Cytokine Growth Factor Rev 2002;13:223–40.

[24] Andoh A, Shioya M, Nishida A, Bamba S, Tsujikawa T, Kim-Mitsuyama S, et al. Expression of IL-24, an activator of the JAK1/STAT3/SOCS3 cascade, is enhanced in inflammatory bowel disease. J Immunol 2009;183:687–95. http://dx.doi.org/10.4049/jimmunol.0804169 [Epub 2009 Jun 17].

[25] Sheikh F, Baurin VV, Lewis-Antes A, Shah NK, Smirnov SV, Anantha S, et al. Cutting Edge: IL-26 signals through a novel receptor complex composed of IL-20 receptor 1 and IL-10 receptor 2. J Immunol 2004;172:2006–10.

[26] Chada S, Bocangel D, Ramesh R, Grimm EA, Mumm JB, Mhashilkar AM, et al. Mda-7/IL24 kills pancreatic cancer cells by inhibition of the Wnt/PI3K signaling pathways: identification of IL-20 receptor-mediated bystander activity against pancreatic cancer. Mol Ther 2005;11:724–33.

[27] Whitaker EL, Filippov VA, Filippova M, Guerrero-Juarez CF, Duerksen-Hughes PJ. Splice variants of mda-7/IL-24 differentially affect survival and induce apoptosis in U2OS cells: Functional characterization of mda-7/IL-24 splice isoforms. Cytokine 2011;56:272–81. http://dx.doi.org/10.1016/j.cyto.2011.07.020 [Epub 2011 Aug 16].

[28] Zhu Y, Du X, Chen H, Xie Y, Sheng W, Yang J. Effect of adenovirus-mediated ING4 and IL-24 co-expression on chemosensitivity to human lung adenocarcinoma in vitro and in vivo. Sheng Wu Gong Cheng Xue Bao 2011;27:85–94.

[29] Tian H, Wang J, Zhang B, Di J, Chen F, Li H, et al. MDA-7/IL-24 induces Bcl-2 denitrosylation and Ubiquitin-degradation involved in cancer cell apoptosis. PLoS One 2012;7(5):e37200. http://dx.doi.org/10.1371/journal.pone.0037200 [Epub 2012 May 21].

[30] Hadife N, Nemos C, Frippiat JP, Hamadé T, Perrot A, Dalloul A. Interleukin-24 mediates apoptosis in human B-cells through early activation of cell cycle arrest followed by late induction of the mitochondrial apoptosis pathway. Leuk Lymphoma 2012 [Epub ahead of print].

[31] Argiris K, Panethymitaki C, Tavassoli M. Naturally occurring, tumor-specific, therapeutic proteins. Exp Biol Med (Maywood) 2011;236:524–36. http://dx.doi.org/10.1258/ebm.2011.011004.

[32] Park MA, Hamed HA, Mitchell C, Cruickshanks N, Dash R, Allegood J, et al. A serotype 5/3 Adenovirus expressing mda-7/IL-24 infects renal carcinoma cells and promotes toxicity of agents that increase Ros and Ceramide levels. Mol Pharmacol 2011;79:368–80. http://dx.doi.org/10.1124/mol.110.069484.

[33] Sauane M, Su ZZ, Dash R, Liu X, Norris JS, Sarkar D, et al. Ceramide plays a prominent role in Mda-7/IL-24-induced cancer-specific apoptosis. J Cell Physiol 2010;222:546–55.

[34] Zhao L, Gu J, Dong A, Zhang Y, Zhong L, He L, et al. Potent antitumor activity of oncolytic adenovirus expressing mda-7/IL-24 for colorectal cancer. Hum Gene Ther 2005;16:845–58.

[35] Mumm JB, Ekmekcioglu S, Poindexter NJ, Chada S, Grimm EA. Soluble human MDA-7/IL-24: characterization of the molecular form(s) inhibiting tumor growth and stimulating monocytes. J Interferon Cytokine Res 2006;26(12):877–86.

[36] Zhang BF, Liu JJ, Pei DS, Yang ZX, Di JH, Chen FF, et al. Potent antitumor effect elicited by RGD-mda-7, an mda-7/IL-24 mutant, via targeting the integrin receptor of tumor cells. Cancer Biother Radiopharm 2011;26(5):647–55. http://dx.doi.org/10.1089/cbr.2011.0984 [Epub 2011 Sep 8].

[37] Fisher PB. Is *mda-7/IL-24* a "Magic Bullet" for Cancer? Cancer Res 2005;65:10128. http://dx.doi.org/10.1158/0008-5472.CAN-05-3127.

[38] Kim DW, Zhao L, Hanover J, Willingham M, Cheng SY. Thyroid hormone receptor β suppresses SV40-mediated tumorigenesis via novel nongenomic actions. Am J Cancer Res 2012;2(5):606–19.

[39] Piri Z, Esmaeilzadeh AR, Hajikhanmirzaei M. Interleukin-25 as a candidate gene in immunogene therapy of pancreatic cancer. JMHI 2012;6(2):75–9.

[40] Mazaheri T, Esmaeilzadeh AR, H.KH Mirzaei M. Introducing the immunomodulatory effects of mesenchymal stem cells in an experimental model of Behçet's disease. JMHI 2012;6(1):223–27.

[41] Whitaker EL, Filippov VA, Duerksen-Hughes PJ. Interleukin 24: mechanisms and therapeutic potential of an anti-cancer gene. Cytokine Growth Factor Rev 2012;23:323–31. http://dx.doi.org/10.1016/j.cytogfr.2012.08.004 [Epub 2012 Sep 14].

[42] Pei DS, Yang ZX, Zhang BF, Yin XX, Li LT, Li HZ, et al. Enhanced apoptosis-inducing function of MDA-7/IL-24 RGD mutant via the increased adhesion to tumor cells. J Interferon Cytokine Res 2012;32(2):66–73. http://dx.doi.org/10.1089/jir.2011.0040 [Epub 2012 Jan 16].

[43] Sahoo A, Jung YM, Kwon HK, Yi HJ, Lee S, Chang S, et al. A novel splicing variant of mouse interleukin (IL)-24 antagonizes IL-24-induced apoptosis. J Biol Chem 2008;283:28860–72. http://dx.doi.org/10.1074/jbc.M802510200 [Epub 2008 Aug 15].

[44] Azab B, Dash R, Das SK, Bhutia SK, Shen XN, Quinn BA, et al. Enhanced delivery of mda-7/IL-24 using a serotype chimeric adenovirus (Ad.5/3) in combination with the Apogossypol derivative BI-97C1 (Sabutoclax) improves therapeutic efficacy in low CAR colorectal cancer cells. J Cell Physiol 2012;227(5):2145–53. http://dx.doi.org/10.1002/jcp.22947.

[45] Fisher PB, Gopalkrishnan RV, Chada S, Ramesh R, Grimm EA, Rosenfeld MR, et al. Mda-7/IL-24, a novel cancer selective apoptosis inducing cytokine gene: from the laboratory into the clinic. Cancer Biol Ther 2003;2:S23–37.

[46] Jiang H, Lin JJ, Su ZZ, Goldstein NI, Fisher PB. Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression. Oncogene 1995;11(12):2477–86.

[47] Jiang H, Su ZZ, Lin JJ, Goldstein NI, Young CS, Fisher PB. The melanoma differentiation associated gene mda-7 suppresses cancer cell growth. Proc Natl Acad Sci USA 1996;93(17):9160–5.

[48] Bosanquet DC, Harding KG, Ruge F, Sanders AJ, Jiang WG. Expression of IL-24 and IL-24 receptors in human wound tissues and the biological implications of IL-24 on keratinocytes. Wound Repair Regen 2012;20:896–903. http://dx.doi.org/10.1111/j.1524-475X.2012.00840.x.

[49] Tamai H, Miyake K, Yamaguchi H, Takatori M, Dan K, Inokuchi K, et al. AAV8 vector expressing IL24 efficiently suppresses tumor growth mediated by specific mechanisms in *MLL/AF4*-positive ALL model mice. Blood 2012;119:64–71. http://dx.doi.org/10.1182/blood-2011-05-354050.

[50] Xuan W, Li YJ, Liu G, Ben-David Y, Archer MC. Interleukin-24 induces expression of β_4 integrin but suppresses anchorage-independent growth of rat mammary tumor cells by a mechanism that is independent of β_4. Mol Cancer Res 2009;7:433–42.

[51] Wang CJ, Xiao CW, You TG, Zheng YX, Gao W, Zhou ZQ, et al. Interferon-α enhances antitumor activities of oncolytic

adenovirus-mediated IL-24 expression in hepatocellular carcinoma. Mol Cancer 2012;11(17):31.

[52] Jen EY, Poindexter NJ, Farnsworth ES, Grimm EA. IL-2 regulates the expression of the tumor suppressor IL-24 in melanoma cells. Melanoma Res 2012 Feb;22(1):19–29. http://dx.doi.org/10.1097/CMR.0b013e32834d2506.

[53] Tirodkar TS, Voelkel-Johnson C. Sphingolipids in apoptosis. Exp Oncol 2012;34(3):231–42.

[54] Sahoo A, Im SH. Molecular mechanisms governing IL-24 gene expression. Immune Network 2012;12(1):1–7.

[55] Dash R, Richards JE, Su ZZ, Bhutia SK, Azab B, Rahmani M, et al. Mechanism by which Mcl-1 regulates cancer-specific apoptosis triggered by mda-7/IL-24, an IL-10-related cytokine. Cancer Res 2010;70:5034–45.

[56] Dent P, Yacoub A, Hamed HA, Park MA, Dash R, Bhutia SK, et al. MDA-7/IL-24 as a cancer therapeutic: from bench to bedside. Anticancer Drugs 2010;21(8):725–31. http://dx.doi.org/10.1097/CAD.0b013e32833cfbe1.