

Review Article

Therapeutic Applications of Interleukin 24 (IL24): A Review

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Abstract

Fisher's group identified melanoma differentiation-associated protein-7 (MDA-7) upon discovery of cell surface receptor MDA-7 renamed Interleukin 24 (IL24). It has three N-glycosylation sites. IL24 signals through receptors. Binding of IL24 to receptors leads to the activation of STAT-3 and STAT-1. IL 24 induces the secretion of high level of Interferon Gamma (IFN- γ), IL6 and tumor necrosis factor alpha (TNF- α) and low levels of IL1, IL12 and granulocyte macrophage colony stimulating factor (GM-CSF) from human peripheral blood mononuclear cells (PBMC). IL24 has growth suppressive properties in a wide variety of human cancer cell lines without inducing harmful effects in normal cells. This review is focused on the role of IL 24 on tumor cell biology and its potential therapeutic applications.

Keywords: Melanoma differentiation, Protein, Therapeutics, Interleukin, N-glycosylation, Cancer.

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INTRODUCTION

In the mid-1990s, Paul Fisher's group at Colombia University identified a gene by subtraction hybridization, MDA-7 (melanoma differentiation-associated protein-7). In 2002 MDA-7 was renamed interleukin 24 (IL24) upon discovery of cell surface receptors [1]. The molecular weight of human IL24 is 23.8Kd. The molecular weight of unglycosylated IL24 is 18 Kd (Table 1), while secreted glycosylated IL24 has a molecular weight of 35 Kd [2]. IL24 contains three N-glycosylation sites ($N_{85}IT$, $N99VS$, and $N_{126}RT$). N-glycosylation plays a critical role in modulating immunological and biological functions of glycoprotein [3]. IL24 genomic locus has been mapped to chromosome 1q32 with an IL10 related gene cluster containing four genes including IL10, IL19 and IL20. IL24 has been designated as member of interleukin 10 family of cytokines. This family includes IL10, IL19, IL20, IL22, IL24 and IL26 [4]. IL24 signals through its corresponding heterodimeric receptors consisting of IL20R1/IL20R2 and IL22R1/IL20R2. The R1 and R2 are receptors subunits, R1 contains a long cytoplasmic tail and R2 contain a short cytoplasmic tail. IL24 has equal affinity for both receptor complexes. Binding of IL24 to both receptors leads to the activation of STAT-3 and to a lesser extent STAT-1 [5]. IL24 receptors are expressed on human neutrophils, CD4⁺ T- cells, CD8⁺ T- cells and murine neutrophils [3]. Expression of IL24 receptor depends on restrictive expression of IL20R2 in certain non-haemopoietic tissues including skin, lungs, testis and ovary [6]. Over expression of IL24 receptor has been reported in the epidermis of psoriatic skin [7]. The expression of IL24 is inversely correlated with melanoma progression. Analysis of IL10 family member mRNA has revealed that IL24 mRNA is expressed in monocytes and T-cells [8]. IL24 over expressed in colorectal cancers with microsatellite instability [9]. IL24 mediates its biological functions through autocrine and paracrine actions. IL 24 induces the secretion of high level of Interferon Gamma (IFN- γ) ,

IL6 and tumor necrosis factor alpha (TNF- α) and low level of IL1,IL12 and granulocyte macrophage colony stimulating factor (GM-CSF) from human peripheral blood mononuclear cells (PBMC) [3].

Table 1: Characteristics of Interleukin 24 [2]

| Parameter | Homodimer and monomer |
|---------------------|---|
| Size (mol wt) | 23.8 kd (predicted size of unprocessed precursor) 18 kd (unglycosylated mature protein) 35 kd (observed size of secreted IL-24, glycosylated) |
| Receptors | IL20R1/IL-20R2 and IL-22R1/IL-20R2 |
| Cell sources | Melanocytes, T cells,monocytes |
| Cell targets | Cancer cells |
| Major functions | Tumor suppression |
| Disease association | Melanoma,Psoriasis |

Melanoma differentiation associated gene-7/IL24 and melanoma

Melanoma represents an aggressive cancer that most frequently metastasizes the lymph node region and to distant sites as the disease progress. In the United States the incidence of melanoma is increasing at a faster rate than any other cancer [10]. Surgery is an option for treating metastases as chemotherapy and radiotherapy do not achieve cure in majority of the patients and less than 5% of melanoma patients with systemic metastases survive 5 years or more[10]. Treatment with inclusion of a high level of Interferon alpha has shown significant increase in patient life time but not act towards the cure [11]. The metastases treatment focus on expression of skin cell protein as IL24 mRNA and protein are expressed in melanocytes and the only skin cell expressing IL24 protein constitutively [12]. Gupta et al [10] reported the expression of IL24 during melanoma progression. The

immune-histochemical analysis of IL24 expression during tissue section of melanoma indicated abundant expression of IL24 protein in human nevi and in primary melanoma tumors. IL24 has growth suppressive properties in wide variety of human cancer cell lines without inducing harmful effects in normal cells [10].

Apoptosis Inducing Properties of IL24

IL24 has growth inhibiting and apoptosis inducing properties in human tumors [13]. IL24 role in inducing apoptosis has been extensively studied since it was identified. The exact mechanism for this protein to act as a tumor suppressor is still unknown. The secreted intracellular IL24 is able to induce apoptosis and inhibit tumor cell invasion [13]. The apoptosis inducing properties of IL24 was first tested using replication incompetent adenoviral vector (Ad.IL24) in breast cancer cell lines (Table 2). Ad.IL24 inhibited growth and decreased the survival of the tumor cells invitro and invivo without having the same effects on normal cells [4]. This apoptosis inducing ability of Ad.IL24 was demonstrated to be independent of the p53 or retinobla-

Table 2: Summary of activities of Ad-md47 and MDA-7 [6]

| Function /activity | Ad-md47 | MDA-7 |
|--------------------------|---------|-------|
| Normal cell cytotoxicity | -- | -- |
| Tumor cell cytotoxicity | ++ | ++ |
| STAT3 activation | ++ | ++ |
| Cytokine induction | ++ | ++ |
| Anti-angiogenesis | ++ | ++ |

stoma (RB) tumor suppressor status of the target cells [14]. In prostate cancer cells free radicals are involved in IL24-induced apoptosis. Ad.IL24 selectively induces apoptosis by mitochondrial dysfunction and production of reactive oxygen species (ROS). IL24 increases the reactive oxygen species (ROS) production by 3-5 fold in cancer cells without having the same effect on normal cells [15]. A number of molecules involved in apoptotic signaling of IL24 in tumor cells have been identified. However identities of the

critical molecules or signaling pathways for tumor cell apoptosis have not been assortmend [6, 16].

Antitumor bystander activity of IL24

The secreted form of IL24 has antitumor bystander activity. The exact mechanism of this activity is not yet known but it is thought to involve direct cytotoxicity, anti-angiogenic activity or it can be result of immunoregulatory activities [17]. IL24 is secreted from both normal and cancer cells after administration of Ad.IL24. These secreted proteins possess the antitumor bystander activity and are able to suppress growth, induce apoptosis and enhance radiation lethality in human cancer cells [18].

Anti-angiogenic activity of IL24

Angiogenesis is complex process that involves the formation of new blood vessels [19]. It is a fundamental event for tumor growth and metastasis. A number of angiogenesis inhibitors have been found and prove to be effective in pre clinical studies but very few of them have therapeutic effects in clinical trials [19]. IL24 secreted from transfected 293 cells inhibits endothelial cells differentiation and tumor growth. The inhibition of tumor growth was observed to be associated with reduced tumor vascularization. IL24 secreted from transfected 293 cells was detected to be more potent in inhibiting endothelial cell differentiation than endostatin. IL24 inhibits the endothelial cell migration [20].

Combination Therapy with IL24

IL24 is able to efficiently induce apoptosis in a wide variety of human cancers except pancreatic and ovarian cancer [21]. IL24 does not significantly alter growth or induce apoptosis in human pancreatic cancer cells due to the expression of oncogenic K-ras, which is a common genetic alteration in pancreatic cancers. This resistance for IL24 can be overcome by combination treatment

with an antisense K-ras expression vector. Another way to overcome the problem is to use IL24 in combination with non-toxic doses of a chemical compound from the endoperoxide class [21]. It has been reported that IL24 is able to induce apoptosis in ovarian cancer cells with a low efficiency. Induction of apoptosis by IL24 can be enhanced by treating ovarian cells with ionizing radiation in combination with Ad.IL24 [11].

IL24 and Mycobacterium tuberculosis

Tuberculosis remains a global health problem. Multi-drug resistance tuberculosis (MDR)-TB, co infection with HIV and limited efficacy of BCG vaccine against TB in epidemic region of the word drew attention to the urgent need for potent anti-TB drug. IL24 shows a protective effect against mycobacterium tuberculosis infection *in vivo*. IL24 acts as immunological regulatory molecule. IL24 receptors are expressed on CD8⁺ T cells. IL24 activate human CD8⁺ T cells, driving CD8⁺ T cells to produce Th1 type cytokine IFN-γ and counteract TB [3].

IL24 in cancer therapy

IL24 has clinical potential as an anti-cancer drug. It has many advantages compared to existing cancer therapeutics. These include activity against broad range of tumor types, lack of cytotoxicity against normal human cells, ability to induce antitumor bystander activity [18], ability to inhibit tumor angiogenesis and synergy with radiation, chemotherapy and monoclonal antibody therapies [8]. IL24 as fusion protein or with combination of viral vector confirm anticancer effects and immune modulatory activity [10].

CONCLUSION

IL24 has progressed from a laboratory discovery to a potential therapeutic agent for Mycobacterium tuberculosis and cancer. Further investigations are clearly required to have a precise understanding of the

mechanism of action of IL24 in cancer. Future research will be focused on the application of nanotechnology and stem cell to deliver IL24 therapeutically.

REFERENCES

1. Caudell EG, Mumm JB, Poindexter N, Ekmekcioglu S, Mhashilkar AM, Yang XH, Retter MW, Hill P, Chada S, Grimm EA. The protein product of the tumor suppressor gene, melanoma differentiation-associated gene 7, exhibits immunostimulatory activity and is designated IL-24. *J Immunol* 2002; 168: 6041-6046.
2. Mübeck A, Simone B, Reto C, Thomas E, Hiroyuki F, Enrique G, Sven K, Norbert M, Liam OM, Oscar P et al. Interleukins, from 1 to 37, and interferon-γ: Receptors, functions, and roles in diseases. *Journal of allergy and clinical immunology*. 2011; 127: 701-721.
3. Yunfeng Ma, Hai-Dan C, Yubin W, Qilong W, Yingying L, Yinglan Z, Xiao-lian Z, Interleukin 24 as a novel potential cytokine immunotherapy for the treatment of Mycobacterium tuberculosis infection. *Microbes and Infection* 2011; 13: 1099-1110.
4. Chada S, Sutton RB, Ekmekcioglu S, Ellerhorst J, Mumm JB, Leitner WW, Yang HY, Sahin AA, Hunt KK, Fuson KL et al. MDA-7/IL-24 is a unique cytokine--tumor suppressor in the IL-10 family. *Int Immunopharmacol* 2004; 4: 649-667.
5. 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: 7341-7347.
6. Chada S, Mhashilkar AM, Ramesh R, Mumm JB, Sutton RB, Bocangel D, Zheng M, Grimm EA, Ekmekcioglu S. Bystander activity of Ad-md7: human mda-7 protein kills melanoma cells via an IL-20 receptor-dependent but STAT3-independent mechanism. *Mol Ther* 2004; 10: 1085-1095.
7. Huang EY, Madireddi MT, Gopalakrishnan RV, Leszczyniecka M, Su Z, Lebedeva IV, Kang D, Jiang H, Lin JJ, Alexandre D et al. Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppressing and apoptosis inducing properties. *Oncogene* 2001; 20: 7051-7063.
8. Wolk K, Kunz S, Asadullah K, Sabat R. Cutting edge: Immune cells as sources and targets of the IL-10 family members. *J Immunol* 2002; 168 (11): 5397- 402.
9. Allen MB, Pratscher F, Roka C, Krepler V, Wacheck C, Schofer H, Pehamberger M, Muller, T Lucas. Loss of novel mda-7 splice

- variant (*mda-7s*) expression is associated with metastatic melanoma. *J Invest Dermatol* 2004; 123: 583-588.
10. Gupta P, Su ZZ, Lebedeva IV, Sarkar D, Sauane M, Emdad L, Bachelor MA, Grant S, Curiel DT, Dent P et al. *Mda-7/IL-24: Multifunctional cancer-specific apoptosis-inducing cytokine*. *Pharmacol Ther* 2006a; 111: 596-628.
 11. Leath CA, Kataran M, Bhagavatula P, Gopalkrishnan RV, Dent P, Fisher PB, Pereboev A, Carey D, Lebedeva IV, Haisma HJ et al. *Infectivity enhanced adenoviral-mediated mda-7/IL-24 gene therapy for ovarian carcinoma*. *Gynecol Oncol* 2004; 94: 352-362.
 12. 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: 2477-86.
 13. Sauane M, Gopalkrishnan RV, Choo HT, Gupta P, Lebedeva IV, Yacoub A, Dent AP, Fisher PB. *Mechanistic aspects of mda - 7/ IL-24 cancer cell selectivity analysed via a bacterial fusion protein*. *Oncogene* 2004; 23: 7679-7690.
 14. Su ZZ, Madireddi MT, Lin JJ, Young CS, Kitada S, Reed JC, Goldstein NI, Fisher PB. *The cancer growth suppressor gene mda-7 selectively induces apoptosis in human breast cancer cells and inhibits tumor growth in nude mice*. *Proc Natl Acad Sci USA* 1998; 95: 14400-14405.
 15. Lebedeva IV, Su ZZ, Sarkar D, Kitada S, Dent P, Waxman S, Reed JC, Fisher PB. *Melanoma differentiation associated gene-7, mda-7/interleukin-24, induces apoptosis in prostate cancer cells by promoting mitochondrial dysfunction and inducing reactive oxygen species*. *Cancer Res* 2003; 63: 8138-8144.
 16. Yacoub A, Mitchell C, Lebedeva IV, Sarkar D, Su ZZ, McKinstry R, Gopalkrishnan RV, Grant S, Fisher PB, Dent P. *Mda-7 (IL-24) Inhibits growth and enhances radiosensitivity of glioma cells in vitro via JNK signaling*. *Cancer Biol Ther* 2003; 2: 347 - 353.
 17. Fisher PB. *Is mda-7/IL-24 a "magic bullet" for cancer?* *Cancer Res* 2005; 65: 10128-10138.
 18. Su Z, Emdad L, Sauane M, Lebedeva IV, Sarkar D, Gupta P, James CD, Randolph A, Valerie K, Walter MR et al. *Unique aspects of mda-7/ IL-24 antitumor bystander activity: establishing a role for secretion of mda-7/ IL-24 protein by normal cells*. *Oncogene* 2005; 24: 7552-7566.
 19. Cristofanilli M, Charnsangavej C, Hortobagyi GN. *Angiogenesis modulation in cancer research: novel clinical approaches*. *Nat Rev Drug Discov* 2002; 1: 415-426.
 20. Ramesh R, Mhashilkar AM, Tanaka F, Saito Y, Branch CD, Sieger K, Mumm JB, Stewart AL, Boquio A, Dumoutier L et al. *Melanoma differentiation - associated gene 7 / interleukin (IL) -24 is a novel ligand that regulates angiogenesis via the IL-22 receptor*. *Cancer Res* 2003; 63: 5105-5113.
 21. Lebedeva IV, Washington I, Sarkar D, Clark JA, Fine RL, Dent P, Curiel DT, Turro NJ, Fisher PB. *Strategy for reversing resistance to a single anticancer agent in human prostate and pancreatic carcinomas*. *Proc Natl Acad Sci USA* 2007; 104: 3484-3489.