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Short Communication Open Access

Immunogenetherapy of Cancer Using Recombinant Adenovirus Expressing Type III interferon IL-28A or IL-29

Muneo Numasaki[1*], Masatoshi Tagawa[2], Hiroki Tsukamoto[3], Yoshihisa Tomioka[3], Takashi Ohrui[4], and Hiroyuki Arai[1]

[1]*Department of Geriatrics and Gerontology, Institute of Development, Aging and Cancer, Tohoku University, Miyagi, Japan*

[2]*Division of Pathology and Cell Therapy, Chiba Cancer Centre Research Institute, Chiba, Japan*

[3]*Laboratory of Oncology, Pharmacy Practice and Sciences, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi, Japan*

[4]*Division of Geriatric Pharmacotherapy, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan*

[*]**Corresponding author:** Muneo Numasaki, Department of Geriatrics and Gerontology, Institute of Development, Aging and Cancer, Tohoku University, Miyagi, Japan, Tel :+81-22-717-7182; E-mail: numasaki77@aol.com

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Short Communication

The discovery that type I interferons (IFNs) (IFN-α and IFN-β) have antitumor actions, including promotion of innate and acquired antitumor immune responses, suppression of proliferation and induction of apoptosis of tumor cells, in addition to their well-known ability to inhibit virus replication, was initially welcomed with great excitement [1]. Actually, type I IFNs represent the cytokines exhibiting the longest record of use in the treatment of certain hematological malignancies such as hairy cell leukemia and chronic myeloid leukemia [2], and in the adjuvant treatment of certain malignancies, including melanoma and renal cell carcinoma [3,4]. On the other hand, severe toxicity associated with type I IFN therapy, resistance to the treatment, and less than optimal responses have restricted its clinical application and reduced its potential as an anticancer agent.

Type I IFNs also play an essential role in cancer immunosurveillance, and the response of type I IFNs is regulated by epigenetic mechanisms. Histone methyltransferase G9a, which is able to establish heterochromatin modification at the IFN-stimulated genes (ISGs) loci, negatively regulates the response of type I IFNs [5]. Additionally, another H3K9 methyltransferase G9a-like protein (GLP), the partner protein of G9a, has also been found to be associated with suppression of ISGs transcription [6]. As G9a and GLP form a complex and play an essential role in the establishment of heterochromatin and maintenance of imprinted DNA methylation [7,8], the existence of G9a and GLP at ISGs loci might also lead to the gain of DNA methylation to some extent. Furthermore, as type I IFN response is critical for anti-tumor processes and aberrant profiling of DNA methylation is strongly associated with tumorigenesis [9,10], it might be a novel direction to investigate the patterns of DNA methylation at the ISGs loci in the initiation and development processes of malignant carcinomas.

With increasing interest in alternative options to type I IFN-based treatments, the recent discovery of a third type of IFNs (type III IFNs) (IFN-λs/interleukin (IL)-29/IL-28), which shares the same biological properties of type I IFNs, opens the door for evaluating the therapeutic potential of type III IFNs as the toxicity of treatment with type III IFN has been expected to be reduced because of the more limited target cell population than type I IFNs. An ensemble of studies has been performed where the type III IFN gene was transduced into different types of mouse tumor cells, and the *in vivo* behaviour of genetically modified cells constitutively secreting type III IFN was evaluated after administration into immunocompetent syngeneic host [11-13]. The growing body of evidence suggests that type III IFNs might mediate indirect antitumor actions via induction of efficient antitumor immunity in addition to direct action toward tumor cells. Although intracellular signaling pathways are shared between type I IFNs and type III IFNs (Figure 1), the mechanisms underlying antitumor action of type III IFNs might be qualitatively different from type I IFNs (Figures 2 and 3). In addition, in contrast to type I IFNs, type III IFNs could cause fewer serious side effects and suitable for certain types of malignancies as not all cells are responsive to this cytokine.

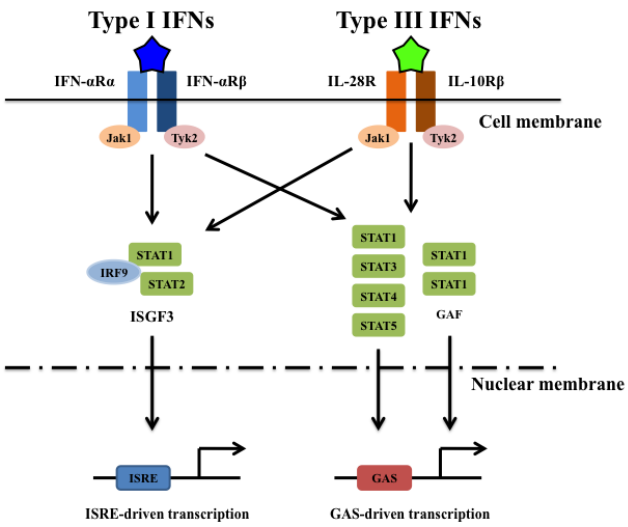

Figure 1: Although type III IFNs use distinct heterodimeric receptor complex consisting of IL-28R and IL-10Rβ from type I IFN receptor complex comprised of either IFN-αRα and IFN-αRβ heterodimer or IFN-αRα homodimer, the downstream intracellular signaling pathways are similar to those mediated by type I IFNs. IRF9: interferon regulatory factor 9, ISGF3: interferon-stimulated gene factor 3, ISRE: interferon-sensitive response element, GAF: interferon-γ activation factor, GAS: interferon-γ-activated site.

Ahmed et al. first evaluated the antitumor activities of type III IFN (IL-28A) using a gene therapy approach instead of systemic therapy [11]. This study provided the initial evidence that tumorigenicity of B16 melanoma producing IL-28A in immunocompetent mice was highly impaired or completely abolished, and that inhibition of tumor establishment was dependent on the amount of IL-28A released by the genetically modified cells [11]. This study also demonstrated that,

using B16 clone insensitive to anti-proliferative effects of IL-28A, host-defense mechanisms played a major role in mediating type III IFN-induced antitumor activity *in vivo*, but failed to develop a strong long-lasting immune memory [11]. In this tumor model, with the findings that infiltrating immune cells were not observed in tumor tissues from B16 cells secreting IL-28A, and primary lymphocytes and macrophages are unresponsive to type III IFNs, it was proposed as one of the possible mechanisms that IL-28A produced by genetically modified cells could first act on neighboring keratinocytes and other tumor stromal cells, and could inhibit their tumor-supportive function, leading to reduced tumorigenicity in the B16 melanoma model [11].

In addition, there was a report demonstrating that transduction of B16/F0 mouse melanoma cells with IL-28A cDNA resulted in *in vitro* cell growth inhibition and increased caspase-3 and caspase-7 activity and $p21^{Waf1/Cip1}$ expression levels, and decreased phosphorylation of Rb [13]. Furthermore, local secretion of IL-28A by B16/F0 cells inhibited to form pulmonary metastasis, largely depending on NK cells [13]. Moreover, targeting of Colon26 liver metastatic lesions by hydrodynamic gene delivery of plasmid DNA encoding mouse IL-28A led to marked reduction of liver metastatic foci along with survival advantages, through pronounced increase of the number of NK cells and NKT cells in the liver [13].

We recently evaluated the immunogenetherapy of cancer using recombinant adenovirus expressing type III IFN, and reported that, despite type III IFN-resistant phenotype, successful induction of antitumor immune responses following multiple administration of recombinant adenovirus expressing human IL-28A (AdIL-28A) or IL-29 (AdIL-29) was subsequently observed in the MCA205 and B16 tumor models (Figure 4) [14]. We observed that *in vivo* growth of MCA205 or B16-F10 tumors injected with either AdIL-28 or AdIL-29 was significantly inhibited through IL-28A- or IL-29-elicited host-mediated immune responses [14]. In these tumor models, tumorigenic behavior of tumors treated with either AdIL-28 or AdIL-29 was compared. The growth of tumors injected with AdIL-28A was slightly slower than that of tumors treated with AdIL-29 [14]. This finding might implicate that the potency of antitumor activity of AdIL-29 might be slightly lower than that of AdIL-28A [14].

In regard to the cellular antitumor mechanisms of AdIL-28A and AdIL-29, the findings in mice selectively depleted of various immune cell populations indicated that $CD8^+$ T cells play an important role in AdIL-28A- or AdIL-29-mediated antitumor immunity in the MCA205 tumor model because the protective effect was partially abolished in $CD8^+$ T cell-depleted animals [14]. Additionally, in the MCA205 and B16 tumor models, treatment with AdIL-28A or AdIL-29 induced more powerful tumor-specific cytotoxic T cells against parental MCA205 cells [14]. This is consistent with the observed dense infiltration of $CD8^+$ T cells into MCA205 or B16F10 tumor tissues. However, primary mouse $CD8^+$ T cells are not expressing IL-28R on the cell surface and are found to be unresponsive to IL-28A treatment [12]. This characteristic of type III IFNs is in clear contrast with that of type I IFNs, which can directly act on T cells [15].

A recent report by Jordan et al. indicated that type III IFN IL-29 influences the cytokine production by Con A-stimulated human T cells, which is isolated from peripheral blood mononuclear cells [16]. IL-28A also displayed the biological function to induce chemokine secretion by mouse lung fibroblasts [12]. Taken together, one possible mechanism, by which IL-28A elicits $CD8^+$ T cell responses, is proposed to be that IL-28A first stimulates $CD8^+$ T cells indirectly through induction of other cytokines and chemokines by surrounding cells including stromal fibroblasts and keratinocytes, and subsequently acts on activated T cells directly [12]. Therefore, the detailed mechanisms underlying this $CD8^+$ T cell-dependent antitumor action of type III IFNs remain to be elucidated.

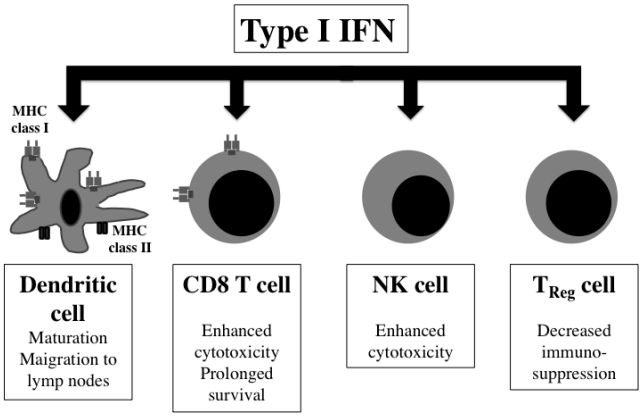

Figure 2: Type I IFNs have a direct effect on the biological activity of various immune cell populations including dendritic cells, $CD8^+$ T cells, NK cells and Treg cells.

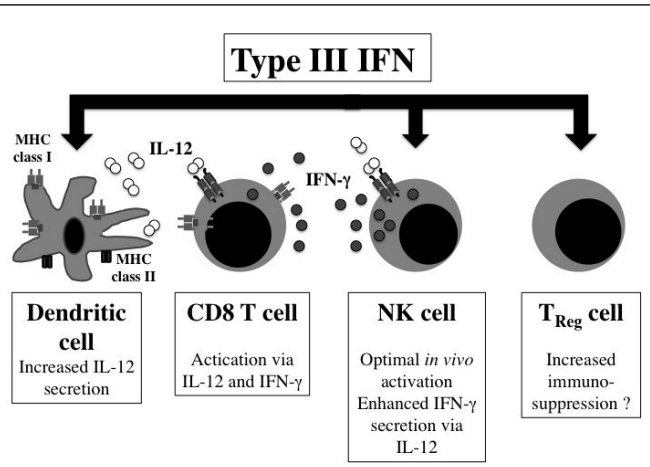

Figure 3: Type III IFNs can directly stimulate dendritic cells to produce biologically active IL-12, which, in turn, stimulates $CD8^+$ T cells, NK cells and Treg cells. NK cells and $CD8^+$ T cells, activated by IL-12, secret large amounts of IFN-γ. In addition, type III IFNs are required for optimal activation of NK cells.

A markedly slower growth rate of MCA205 tumors treated with AdIL-28A in $CD4^+$ T cell-depleted mice was consistently observed, implicating that $CD4^+$ T cells rather inhibit IL-28A-induced antitumor responses [14]. Both $CD4^+$ T cells and $CD8^+$ T cells have been described to be important for the efficient induction of antitumor cellular immunity [17,18]. This unexpected finding that $CD4^+$ T cells are not required for the antitumor activity of IL-28A is not in agreement with the notion that $CD4^+$ T cell help is necessary for the full activation of naive $CD8^+$ T cells [19]. However, a similar inhibitory effect of $CD4^+$ T cells has been previously reported in the IL-12- or IL-23-transduced CT26 tumor model [20,21]. These findings may be possibly explained by taking into account the $CD4^+CD25^+$ T

regulatory cells [22]. With regard to the relation between type III IFNs and $CD4^+CD25^+$ T regulatory cells, Mennechet et al. reported that IL-28A promotes the generation of partially mature dendritic cells (DCs), which display a tolerogenic phenotype [23]. Namely, type III IFN-matured DCs with the ability to migrate lymph nodes express high levels of MHC class I and class II, but low levels of co-stimulatory molecules [23]. These type III IFN-treated DCs specifically induced IL-2-dependent proliferation of a $CD4^+CD25^+Foxp3^+$ T regulatory cell population in culture, which is thought, in general, to result in suppression of the antitumor immune responses [22]. Therefore, type III IFN-treated DCs can stimulate the proliferation of pre-existing $CD4^+CD25^+$ T regulatory cells in the presence of IL-2 and inhibit efficient antitumor immunity. Of particular interest, these findings provide another important evidence that type I IFNs and type III IFNs can exert distinct biologic effects on DC differentiation, phenotype and function.

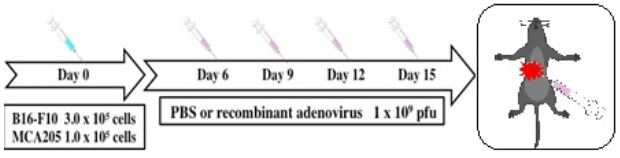

Figure 4: Mice were inoculated with either B16-F10 or MCA205 cells on day 0 followed by the intratumoral injection of PBS, AdNull, AdIL-28A or AdIL-29 on days 6, 9, 12, and 15. (pfu: plaque-forming unit)

The finding that, in the MCA205 tumor model, AdIL-28A-elicited antitumor responses were partially abrogated in NK cell-depleted mice strongly implied that NK cells play an important role in the antitumor activity of AdIL-28A [14]. This finding is consistent with the result demonstrated by Sato et al. using the tumor model of B16 melanoma [13]. However, the surprising finding is that IL-28A is unable to directly enhance NK cell cytolytic activity both *in vitro* and *in vivo* [14]. This biological feature of type III IFNs on NK cells is in sharp contrast with type I IFNs, which markedly promote NK cell-mediated cytotoxicity in culture and *in vivo* [24]. In addition, IL-28A does not have capability to directly stimulate the growth of NK cells in culture [12]. On the other hand, IL-28A administration into SCID mice significantly expanded the splenic NK cells depending on the dose of administration, and expression of IL-28A in the liver increased the hepatic NK cells [12,13]. In the case of type I IFNs, exposure to type I IFNs is closely associated with NK cell blastogenesis and proliferation, but not IFN-γ expression *in vivo* [19]. The immunoregulatory effect of type I IFNs to elicit the expression of IL-15 in mouse cell populations has been proposed to contribute to the induction of NK cell proliferation [20]. In contrast, the detailed mechanisms for type III IFN-induced proliferation of NK cells *in vivo* remain largely unknown [12,13]. Nonetheless, type III IFNs appear to augment NK cell-mediated *in vivo* antitumor activity via increasing the total number of NK cells [12]. Another possible explanation of underlying mechanisms is that IL-28A, like IL-21 [25], could enhance the cytolytic activity of NK cells previously activated by stimulators such as other cytokines and chemokines, but could not induce cytotoxic activity in resting NK cells. Recently, Abushahba et al. reported that DCs are involved in type III IFN-elicited NK cell activation [26]. DCs stimulated by type III IFNs secreted more amounts of bioactive IL-12, which subsequently activated NK cells [27]. Thus, there is a possibility that type III IFNs activate NK cells indirectly via DCs stimulation.

The fact that, in IFN-γ KO mice, the antitumor effects of therapy using AdIL-28A or AdIL-29 were completely abrogated indicated that IFN-γ is critically involved in AdIL-28A- and AdIL-29-mediated antitumor immune responses [14]. IFN-γ is a pleiotropic cytokine that can act on both tumor cells and host immunity [28,29]. IFN-γ directly inhibits proliferation of some tumor cells and indirectly suppresses tumor growth *in vivo* by activating NK cells and macrophages and inducing angiostatic chemokines such as MIG and IP-10 with consequent inhibition of tumor angiogenesis [30,31].

IFN-γ is essentially involved in AdIL-28A-mediated antitumor action, whereas a wide range of doses of IL-28A exert no direct effects on the release of IFN-γ by NK cells and $CD8^+$ T cells stimulated with or without anti-mouse CD3 mAb in culture [12]. In contrast, IL-28A induces IFN-γ release by primary $CD4^+$ T cells stimulated with anti-mouse CD3 mAb or co-stimulated with anti-mouse CD3 mAb plus anti-mouse CD28 mAb [32]. This biological effect of IL-28A on IFN-γ secretion appears to be dose-dependent [32]. In addition, this ability of IL-28A to induce IFN-γ secretion by $CD4^+$ T cells is T-bet dependent [32]. However, in contrast with IL-12, daily administration of IL-28A into C57BL/6 mice for 3 consecutive days could not induce measurable serum IFN-γ levels [12]. Therefore, the pathway from type III IFNs to IFN-γ expression in mice remains to be elucidated.

Combination therapy with AdIL-28A and systemic administration of IL-12 protein has a synergistic antitumor effect without apparent deleterious side effects, suggesting possible advantages in this combined therapy [14]. As mentioned above, type III IFNs themselves appear to have, if any, a limited capability to stimulate IFN-γ secretion in mouse system, whereas type III IFNs significantly enhance IL-12-mediated IFN-γ secretion by $CD4^+$ T cells stimulated with anti-mouse CD3 mAb *in vitro*, and increases serum IFN-γ concentration and the total number of spleen cells as compared with IL-12 alone in C57BL/6 mice [12,32]. This biological effect of type III IFNs on IL-12-induced IFN-γ expression is common with that of type I IFNs. There was a report describing that type I IFN has a modest effect on IL-12 induction of IFN-γ production by mouse cells in culture [33]. Thus, the enhancement of the antitumor effect by combination therapy of AdIL-28A and IL-12 appears to be, at least in part, dependent on increased IFN-γ production [14].

In conclusion, local production of type III IFN using recombinant adenovirus expressing IL-28A or IL-29 at the tumor site is effective in inducing specific antitumor immune responses against tumors. The immune responses elicited by AdIL-28A are dependent upon NK cells and $CD8^+$ T cells, and IFN-γ production. Immunogenetherapy using recombinant adenovirus expressing type III IFN may be a promising therapeutic strategy for cancer.

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Competing Interests

The authors declare that we have no competing interests.

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