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MOLECULAR CELL BIOLOGY

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Antitumor Effect of mIFN-λ3 in C57BL/6 Mice Model for Papilloma Tumors[1]

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Abstract—Although several years have passed since the determination of the human papilloma virus (HPV) as the causative agent for cervical cancer, a definitive treatment has not yet been found. Interferon-alpha (IFN-α) immunotherapy is one of the promising methods for tumor treatment, although numerous side effects were observed in clinical trials. Recently, a new type of interferon, lambda-interferon (IFN-λ), has been discovered with fewer side effects than IFN-α since its receptor repertoire is limited. IFN-λ has a series of activities including antiviral, anti-proliferative and anti-tumor actions. In the present study, the effects of IFN-α and IFN-λ on the TC1 papilloma tumor model in C57BL/6 mice were evaluated. TC1 cells were injected into the mice subcutaneously. Upon tumor formation, murine IFN, mIFN-α and mIFN-λ, expression plasmids were injected intratumorally in combination or alone. The survival time and tumor size as well as apoptosis in tumors and NK cytoxicity were measured after three injections. As compared with the control group, the remarkable results especially in the group which received mIFN-α and mIFN-λ together were obtained for all of the measured parameters. Although IFN-λ is a new member of the interferon family and its properties should be studied in detail, the data obtained suggests that the use of IFN-λ especially in combination with IFN-α could be considered as an effective strategy for papilloma cervical cancer immunotherapy.

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Keywords: interfron-alpha, interferon-lambda, immunotherapy, papilloma tumor

INTRODUCTION

Cervical carcinoma is the second most common form of cancer and one of the most important causes of death among women around the world [1]. Despite numerous studies, treatment of cervical cancer has remained a considerable problem, especially in developing countries [2]. Human papilloma virus (HPV) is the major cause of cervical cancer. More than 150 kinds of papilloma virus biotypes have been known which are divided into genotypes of low, intermediate and high risks based on the ability to induce oncogenesis [3–5]. Despite conventional treatments like cryosurgery, radiation and chemotherapy, a considerable percent of patients continues to die from this disease [6]. There is therefore a need for development of novel treatment strategies [7, 8]. Several antiviral and immunomodulatory agents were evaluated for treatment of HPV-associated cervical tumors [9]. Using the immune system to fight cancer is the basis of biological treatment methods. Nowadays, interferons (IFNs) are used with chemotherapy in biological treatments in order to strengthen the immune system. Furthermore, several biological functions of IFNs, including enhancement of the immune response by activating certain immune cells such as natural killer cells and dendritic cells, the antiangiogenic and proapoptotic effects, make them suitable candidates for anti-cancer therapy [10–12].

On the one hand, the HPV-16 protein E7 was shown to sensitize malignant cells to IFN-α apoptosis. On the other hand, IFNs, such as IFN-α, IFN-β, and IFN-γ, suppress the *E6* and *E7* gene transcripts in certain carcinoma cell lines [13]. The modulatory effect of IFNs on papilloma tumor cells designated them as promising therapeutic agents along with other strategies for HPV tumors [14]. Among the different types of IFNs, IFN-α is the most widely used in spite of the adverse effects induced, namely: nervous system disorders, myelosuppression, flu-like symptoms such as tremble, fever, muscular aches, weakness, loosing appetite, nausea, diarrhea and vomiting that limit its application in clinical stages [10]. During recent years, a new member of the IFN family named interferon type III, or IFN-λ, which was identified from the human genomic sequence was classified as a member of the cytokine type 2 family [15, 16]. This IFN displayed antiviral and antitumor features [17–23]. Sato et al. [21] demonstrated the antitumor effect of IFN-λ in the murine B16 melanoma and Colon 26 tumors. They showed that NK cells were critical for IFN-λ-

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Diffferently treated groups of mice and injection protocol

Group	Material injected	The number of mice	Injection dose, μg/mL	Interval between injections, days	The number of injections
Control	PBS	5	100	14	3
Testifier	pcDNA3.1	5	100	14	3
IFN-λ	pEF-mIFN-λ3	5	100	14	3
IFN-α	pCMV mIFN-α1	5	100	14	3
IFN-α/λ	pEF-mIFN-λ3 + pCMV mIFN-α1	5	50 + 50	14	3

mediated tumor growth inhibition in vivo. The overexpression of IFN-λ in B16 cells induced cell surface MHC class I and Fas (CD95) expression and moreover, markedly increased caspase-3/7 activity concomitant with cell cycle arrest and apoptosis [21]. IFN-λ has similar signaling to that of IFN-α [24–26]. Because of the limited number of receptors and causing naturally less harm, the IFN-α is considered as a promising agent for cancer therapy [10, 26–28]. Here, the antitumor activity of IFN-λ3 was compared with that of IFN-α in papilloma tumors in a mouse model.

EXPERIMENTAL

Plasmids. The eukaryotic expression vector of murine IFN-λ3 (mIFN-λ3) was kindly provided as a gift from Dr. Sergio Kotenko [27], and the expression vector of murine IFN-α1 (mIFN-α1) was dedicated by Dr. Stefano Indraccolo [29]. The expression of both IFNs in eukaryotic cells was confirmed by respective researchers. To confirm the integrity of the vectors, enzyme digestion with XbaI and BamH I of the pCMV mIFN-α1 plasmid and EcoRI and KpnI of the pEF-mIFN-λ3 plasmid was performed. For plasmid injections into mice, the plasmids were extracted using the plasmid DNA purification kit (Intron Biotechnology, Korea).

Cells. The TC1 cell line which expresses the HPV-16E7 and E6 oncoproteins, was used to create tumors in the mouse animal model [30, 31]. The TC1 cells were cultured in DMEM containing 5% of FBS medium. The amount of 1×10^6 cells of the third passage were injected into each mouse. The erythroleukemic cell line K562 was maintained in RPMI-1600 and used as target cells for the NK cell assay.

Animal study. Female C57BL/6 mice (5 weeks old) were purchased from the Pasteur Institute of Iran. The mice were housed for one week before starting the experiment, and maintained in a good condition. All experiments were done according to the Animal Care and Use Protocol of Tarbiat Modares University. To create tumors, 1×10^6 cells were injected to the left or right flank of each mouse. Two weeks after the TC1 injection, when the size of tumors reached about 5 mm, the randomized groups of 5 animals received 3 intratumoral injections at 14-day intervals as described in the table.

The weight of the mice and the size of the tumors were recorded carefully at each step of the experiment.

NK cell cytotoxicity assay. The lytic activity of NK cells was assayed against the K562 cell line, as a highly sensitive target for the NK cells [32]. Fourteen days after the last injection, spleens were aseptically removed and homogenized in complete RPMI-1600 medium. The viability of splenocytes was determined by trypan blue (0.4% w/v) exclusion, and the cells were used as effector ones.

The K562 target cells were distributed into a 96-well plate (2×10^5 cells/well) in 50 μL of RPMI-1600 with 1% serum. The effector cells were added to the target cells at ratios of 25 : 1, 50 : 1 and 100 : 1 for a standard lactate dehydrogenase (LDH) release assay. Wells containing 50 μL of K562 cells plus 50 μL of medium with 1% serum served as the "low" control (lC), with less LDH release, while the "high" control (hC) contained 50 μL of K562 cells plus 50 μL of medium with 1% serum with lysis solution (which was added at the end of the experiment). In the control well with the effector cells (efC), 50 μL of the medium with 1% serum plus 50 μL of spleen cells (the highest density was used) were poured. All experiments were performed in triplicate.

The plates were incubated for 6 to 8 h at 37°C with 90% humidity and 5% CO_2. Then 100 μL of a freshly prepared reaction mixture (LDH Cytotoxicity Detection Kit Plus, Roche, Germany) were added to each well and incubated for 30 min in the dark. Finally, by adding 50 μL of the stop-solution, the reaction was stopped, and optical density was read at the wavelength of 492 nm (A_{492}). The percentage of cytotoxicity was calculated with the following formula:

$$\text{Percent Specific Release (\%)} = 100 \times (A_{ef} - A_{tar} - A_{efC})/(A_{hC} - A_{lC}),$$

where index A is A_{492} value for for effector (ef), target (tar) cells and control cells (see above). The median value for each triplicate was used for the calculations of the cytotoxicity value.

Apoptosis assay. DNA fragmentation was determined by using the In Situ Cell Death Detection kit (Roche, Germany) according to the manufacturer's

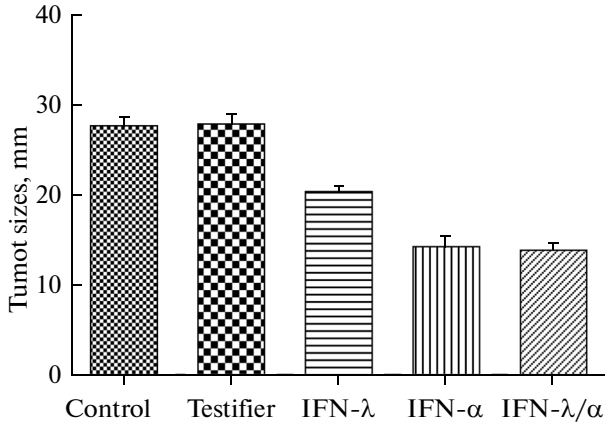

Fig. 1. The mean tumor size 14 days after the last injection.

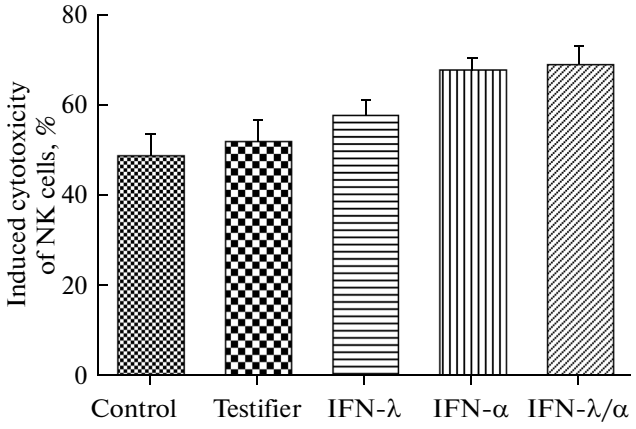

Fig. 2. Cytotoxicity of NK cells. The cytotoxic capacity of splenic NK cells derived from treated mice was significantly higher than that for the control groups ($p < 0.05$). Data on the cytotoxicity induced by different treatments are presented as mean ± SD calculated from 3 experiments.

protocol on de-paraffinized tumor sections, and then the samples were counter stained with hematoxylin and eosin (H&E). The total number of cells in a given area was determined by using DAPI staining. The apoptotic index was determined as the number of TUNEL positive stained cells divided by the total cell number. Three different sections from the center of the tumor were considered for each sample.

Statistical analysis. Data obtained from the size of tumor, NK cell cytotoxicity, apoptosis assay, body weight and survival time were analyzed by one-way ANOVA followed by SPSS software version 19. Values of $p < 0.05$ were considered to be significant.

RESULTS

Measuring the Size of the Tumor

The average size of tumors on the first day of IFN injection was 5 mm. Two weeks after the last injection the sizes of the tumors were measured using a digital caliper (Fig. 1). According to the accomplished calculations, the average size of tumors in the control groups of PBS or empty vector was not statistically different. The mice received IFN-λ had a significantly smaller tumor sizes as compared to the controls (Fig. 1). The injection of IFN-α as well as the combined treatment with IFN-α and IFN-λ resulted in a reduction in tumor size as compared to the IFN-λ group (Fig. 1).

IFN Injection Increased the NK Cell Cytotoxicity

To verify that interferon therapy resulted in an increased cytotoxic potential of NK cells, the splenic cells isolated from treated mice were co-cultured with K562 target cells at different effector/target ratios. These cells have a low MHC expression, which makes them sensitive to NK cell killing. Figure 2 shows that NK cells from control mice were unable to efficiently lyse the target cells. In contrast, target cells were efficiently killed by NK cells from IFN-α and IFN-λ treated mice. These data indicate that IFN treatment strongly improves NK cell cytotoxicity in vitro. Furthermore, the mice injected with a combination of IFN-α and IFN-λ showed a significantly higher specific lysis compared to those of treated with IFN-λ alone ($p < 0.05$)

IFN-Induced Apoptosis in Tumors

Cell apoptosis was measured by the TUNEL assay using the In Situ Cell Death Detection kit 14 days after the last injection. The results revealed the existence of apoptotic cells in IFN-λ, IFN-α and IFN-λ/α groups what indicates the induction of apoptosis in tumors upon IFN therapy (Fig. 3).

Apoptosis was quantified by determining the percentage of biotinylated-dUTP (bdUTP)-positive cells in a minimum of 1000 tumor cells counted in 10 fields of 3 different sections from the center of the each tumor (Fig. 4).

Body Weight and Survival Time

The time of survival was recorded for 7 days after the last injection. Because of the large size of tumors in the 12th weeks of the injection, the both control groups, received PBS and PCDNA3.1+, were scarified. In IFN-α group in the 11th week, 48 h after injecting the second portion, 1 mouse died that was possibly because of the side effect of IFN-α. In IFN-λ and in IFN-λ/α groups no death was observed through 16 weeks. Mice body weights were recorded carefully upon arrival to the lab in the 5th week of their life, at the beginning of the experiment (6th week) and 2 weeks after the first injection (8th week) and also 7 days after the last injection. Naturally, average weight of female C57BL/6 is between 16.9 to 20.7 g at 5 to

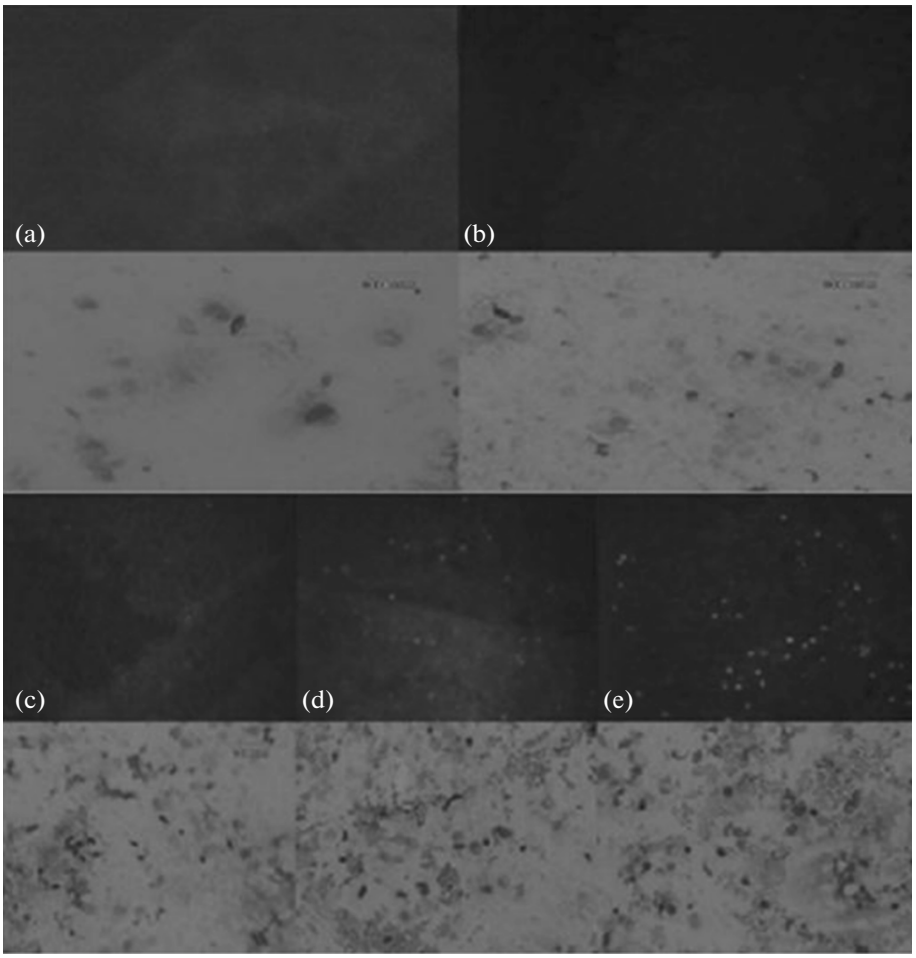

Fig. 3. Tunnel test in tumor sections of mice from different groups: control (a), testifier (b), IFN-λ (c), IFN-α (d), and IFN-λ/α (e). The sections were stained with florescence (up) or DAB (bottom) conjugated antibodies. (The color variant is presented in the Supplement to the article, see site www. molecbio.com.)

12 weeks [33] The average weight of animals in each group at different time points was shown in Fig. 5.

DISCUSSION

As the new member of the cytokine type 3 family, INF-λ, was shown to have antiviral, antitumor and immune regulatory activities [17, 28, 34]. IFN-λ and IFN-α activate an identical group of genes. The restricted amount of receptors of type III IFNs as compared to type I allows us to consider them as possible alternative agents for IFN therapy with fewer side effects [10]. Depending on the tumor type, a great variability was reported in sensitivity to IFN therapy, especially in the case of INF-λ. In the present study, an antitumor activity of IFN-λ alone or in combination with IFN-λ in TC1 papilloma tumor cells were investigated in the C57BL/6 mice model. The TC1 cell line was immortalized with E6 and E7 of the papilloma virus. These oncoproteins were shown to make cells sensitive to IFN-induced apoptosis. This property may depend on the activation of proapoptotic Bak that is down regulated in E6 and E7 transfected cells [13, 14]. It was reported that the TC1 cells originated from C57BL/6 lungs express the IFN-λ3 receptor [35, 36].

In this study, the induction of apoptosis upon IFN administration was shown. The chosen sections from the center of the tumors with uniform cells partly confirmed to cause apoptosis of tumor cells and not of infiltrated lymphocytes which are usually localized around blood vessels. Our findings are in accordance with the data reported by Sato at al. [21] on IFN-λ induced apoptosis in murine B16 melanoma and Colon26 cancer cells transduced with mouse IFN-λ. As achieved in previous studies, type III IFNs improved the therapeutic efficacy of IFN-α. In our study, the combined administration of IFN-λ and IFN-α displayed higher apoptotic death levels and effective inhibitory action on tumor growth than individual IFNs. This regiment reduces the dose and side effects of IFN-λ administration [37, 38]. Although, IFN-α alone also showed a dramatic effect on tumor size reduction and apoptosis induction in comparison

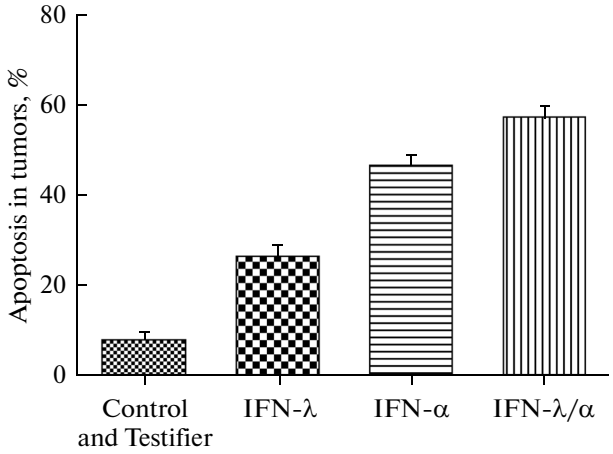

Fig. 4. IFN induced apoptosis in tumors. The highest level of apoptosis was detected in IFN-λ/α group and the lowest one was in control and testifier groups.

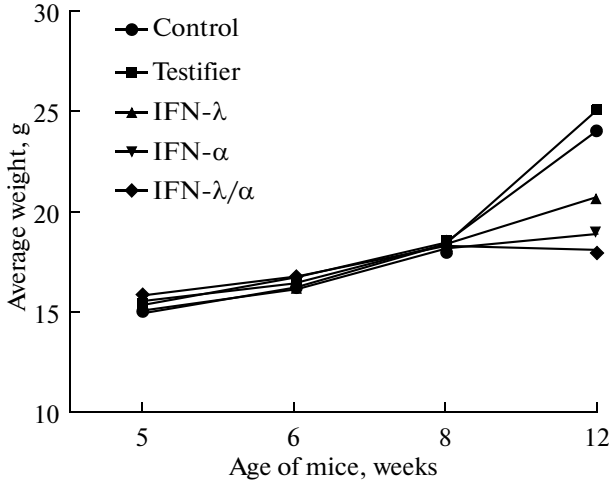

Fig. 5. The average body weight of tumor-bearing mice at different time points. The weight differences among interferon treated and control groups are partly due to the weight of the tumors.

with the control group, IFN-α therapy was more effective in these issues but the death of a mouse in this group may be regarded as a side effect of IFN-α administration.

In addition to the direct apoptosis of tumor cells, IFNs can elicit antitumor activity through the induction of the immune response. It was proved that NK cells are the most important cells mediating in vivo antitumor activity of IFN-λ [21]. IFN-λ stimulates and enhances the number and cytotoxic activity of NK cells [21, 39]. Similarly, using the NK cell cytotoxicity assay we demonstrated that the antitumor activity induced by IFN-λ was mediated by NK cells. All three groups, which received IFN alone or in combination showed statistically significant differences in NK cells cytotoxicity as compared with the control groups and more interestingly, in the IFNs combined group the highest NK cells activity was detected. In summary, this study revealed that IFN-α has an antitumor activity against the papilloma cervical cancer model through induction of apoptosis and enhancement of the NK cells activity. The combination of IFN-α with IFN-λ showed a greater therapeutic effect and thus may be considered as an alternative to IFN-λ immunotherapy in the treatment of papilloma tumors. At present, IFN-α is being investigated to be used for curing some maladies such as hepatitis C [40], but there is still very limited amount of data on the properties IFN-λ what demands more studies to evaluate its potential as a remedial agent in the treatment of tumors. The use of new agents in clinical phases needs more investigations on its effects, route of administration and application of a more safe form of the drug.

REFERENCES

1. de Weerd N.A., Samarajiwa S.A., Hertzog P.J. 2007. Type I interferon receptors: Biochemistry and biological functions. *J. Biol. Chem*. **282**, 20053–20057.
2. TjalmaW.A., van Waes T.R., van den Eeden L.E., Bogers J.J. 2005. Role of human papillomavirus in the carcinogenesis of squamous cell carcinoma and adenocarcinoma of the cervix. *Best Pract. Res. Clin. Obstet. Gynaecol*. **19**, 469–483.
3. Carter J.R., Ding Z., Rose B.R. 2011. HPV infection and cervical disease: A review. *Aust. N. Z. J. Obstet. Gynaecol*. **51**, 103–108.
4. Arbyn M., Castellsagué X., de Sanjosé S., Bruni L., Saraiya M., Bray F., Ferlay J. 2011. Worldwide burden of cervical cancer in 2008. *Ann. Oncol*. **22**, 2675–2686.
5. Anorlu R.I. 2008. Cervical cancer: The sub-Saharan African perspective. *Reprod. Health Matters*. **16**, 41–49.
6. Monk B.J., Mas Lopez L., Zarba J.J., Oaknin A., Tarpin C., Termrungruanglert W., Alber J. A., Ding J., Stutts M.W., Pandite L.N. 2010. Phase II, ppen-label study of pazopanib or lapatinib monotherapy compared with pazopanib plus lapatinib combination therapy in patients with advanced and recurrent cervical cancer. *J. Clin. Oncol*. **28**, 3562–3566.
7. Michel N., Öhlschläger P., Osen W., Freyschmidt E.J., Guthöhrlein H., Kaufmann A.M., Müller M., Gissmann L. 2002. T cell response to human papillomavirus 16 E7 in mice: Comparison of Cr release assay, intracellular IFN-γ production, ELISPOT and tetramer staining. *Intervirology*. **45**, 290–299.
8. Leachman S.A., Shylankevich M., Slade M.D., Levine D., Sundaram R. K., Xiao W., Bryan M., Zelterman D., Tiegelaar R.E., Brandsma J.L. 2002. Ubiquitin-fused and/or multiple early genes from cottontail rabbit papillomavirus as DNA vaccines. *J. Virol*. **76**, 7616–7624.
9. Lin C.T., Wang C.N., Lai C.H. 2013. Immunotherapy for advanced or relapsed cervical cancer. *Gynecol. Mini. Invas. Ther*. **2**, 3–7.
10. Lasfar A., Abushahba W., Balan M., Cohen Solal K.A. 2011. Interferon lambda: A new sword in cancer immunotherapy. *Clin. Dev. Immunol*. **2011**, 349575.
11. Takaoka A., Hayakawa S., Yanai H., Stoiber D., Negishi H., Kikuchi H., Sasaki S., Imai K., Shibue T., Honda K., Taniguchi T. 2003. Integration of interferon-α/β signalling to p53 responses in tumour suppression and antiviral defence. *Nature*. **424**, 516–523.

12. Le Bon A., Tough D.F. 2002. Links between innate and adaptive immunity via type I interferon. *Curr. Opin. Immunol*. **14**, 432–436.
13. Thyrell L., Sangfelt O., Zhivotovsky B., Pokrovskaja K., Wang Y., Einhorn S., Grandér D. 2005.The HPV-16 E7 oncogene sensitizes malignant cells to IFN-alpha-induced apoptosis. *J. Interferon Cytokine Res*. **25**, 63–72.
14. Kim K.Y., BlattL., Taylor M.W. 2000. The effects of interferon on the expression of human papillomavirus oncogenes. *J. Gen. Virol*. **81**, 695–700.
15. Sheppard P., Kindsvogel W., Xu W., Henderson K., Schlutsmeyer S., Whitmore T.E., Kuestner R.,Garrigues U., Birks C., Roraback J., Ostrander C., Dong D., Shin J., Presnell S., Fox B., et al. 2003. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat. Immunol*. **4**, 63–68.
16. Kotenko S.V., Gallagher G., Baurin V.V., Lewis Antes A., Shen M., Shah N.K., Langer J.A., Sheikh F., Dickensheets H., Donnelly R.P. 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol*. **4**, 69–77.
17. Kotenko S.V. 2011. IFN-λs. *Curr. Opin. Immunol*. **23**, 583–590.
18. Li M., Chen Y., Huang T., Liu Y., He S. 2006. Production, characterization, and applications of two novel monoclonal antibodies against human interleukin-28A. *Tissue Antigens*. **68**, 477–482.
19. Numasaki M., Tagawa M., Iwata F., Suzuki T., Nakamura A., Okada M., Iwakura Y., Aiba S., Yamaya M. 2007. IL-28 elicits antitumor responses against murine fibrosarcoma. *J. Immunol*. **178**, 5086–5098.
20. Dumoutier L., Tounsi A., Michiels T., Sommereyns C., Kotenko S.V., Renauld J.C. 2004. Role of the interleukin (IL)-28 receptor tyrosine residues for antiviral and antiproliferative activity of IL-29/Interferon-λ1: Similarities with type I interferon signaling. *J. Biol. Chem*. **279**, 32269–32274.
21. Sato A., Ohtsuki M., Hata M., Kobayashi E., Murakami T. 2006. Antitumor activity of IFN-λ in murine tumor models. *J. Immunol*. **176**, 7686–7694.
22. Brand S., Beigel F., Olszak T., Zitzmann K., Eichhorst S.T., Otte J.M., Diebold J., Diepolder H., Adler B., Auernhammer C.J., Göke B., Dambacher J. 2005. IL-28A and IL-29 mediate antiproliferative and antiviral signals in intestinal epithelial cells and murine CMV infection increases colonic IL-28A expression. *Am. J. Physiol. Gastrointest. Liver Physiol*. **289**, G960–G968.
23. Bartlett N.W., Buttigieg K., Kotenko S.V., Smith G.L. 2005. Murine interferon lambdas (type III interferons) exhibit potent antiviral activity in vivo in a poxvirus infection model. *J. Gen. Virol*. **86**, 1589–1596.
24. Maher S.G., Sheikh F., Scarzello A.J., Romero Weaver A.L., Baker D.P., Donnelly R.P., Gamero A.M. 2008. IFN-α and IFN-λ differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol. Ther*. **7**, 1109–1115.
25. Zitzmann K., Brand S., Baehs S., Göke B., Meinecke J., Spöttl G., Meyer H., Auernhammer C.J. 2006. Novel interferon-λs induce antiproliferative effects in neuroendocrine tumor cells. *Biochem. Biophys. Res. Commun*. **344**, 1334–1341.
26. Tagawa M., Kawamura K., Li Q., Tada Y., Hiroshima K., Shimada H. 2011. A possible anticancer agent, type III interferon, activates cell death pathways and produces antitumor effects. *Clin. Dev. Immunol*. **2011**, 6.
27. Lasfar A., Lewis Antes A., Smirnov S.V., Anantha S., Abushahba W., Tian B., Reuhl K., Dickensheets H., Sheikh F., Donnelly R.P., Raveche E., Kotenko S.V. 2006. Characterization of themouse IFN-λ ligand-receptor system: IFN-λs exhibit antitumor activity against B16 melanoma. *Cancer Res*. **66**, 4468–4477.
28. Li M., Liu X., Zhou Y., Su S.B. 2009. Interferon-λs: The modulators of antivirus, antitumor, and immune responses. *J. Leukoc. Biol*. **86**, 23–32.
29. Calabrò M.L., Gasperini P., Di Gangi I. M., Indraccolo S., Barbierato M., Amadori A., Chieco Bianchi L. 2009. Antineoplastic activity of lentiviral vectors expressing interferon-α in a preclinical model of primary effusion lymphoma. *Blood*. **113**, 4525–4533.
30. Li J., Sun Y., Garen A. 2002. Immunization and immunotherapy for cancers involving infection by a human papillomavirus in a mouse model. *Proc. Natl. Acad. Sci. U. S. A*. **99**, 16232–16236.
31. Ji H., Chang E.Y., Lin K.Y., Kurman R.J., Pardoll D.M., Wu T.C.1998. Antigen-specific immunotherapy for murine lung metastatic tumors expressing human papillomavirus type 16 E7 oncoprotein. *Int. J. Cancer*. **78**, 41–45.
32. Fujisaki H., Kakuda H., Shimasaki N., Imai C., Ma J., Lockey T., Eldridge P., Leung W.H., Campana D. 2009. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. *Cancer Res*. **69**, 4010–4017.
33. Body weight information, JAX mice strain C57BL/6J. 2014. The Jackson Laboratory. http://jaxmice.jax.org/support/weight/000664.html.
34. Abushahba W., Balan M., Castaneda I., Yuan Y., Reuhl K., Raveche E., Torre A., Lasfar A., KotenkoS. 2010. Antitumor activity of type I and type III interferons in BNL hepatoma model. *Cancer Immunol. Immunother*. **59**, 1059–1071.
35. Ank N., West H., Bartholdy C., Eriksson K., Thomsen A.R., Paludan S.R. 2006. Lambda interferon (IFN-λ), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. *J. Virol*. **80**, 4501–4509.
36. Mordstein M., Neugebauer E., Ditt V., Jessen B., Rieger T., Falcone V., Sorgeloos F., Ehl S., Mayer D., Kochs G., Schwemmle M., Günther S., Drosten C., Michiels T., Staeheli P. 2010. Lambda interferon renders epithelial cells of the respiratory and gastrointestinal tracts resistant to viral infections. *J. Virol*. **84**, 5670–5677.
37. Cascinelli N., Belli F., MacKie R.M., Santinami M., Bufalino R., Morabito A. 2001. Effect of long-term adjuvant therapy with interferon alpha-2a in patients with regional node metastases from cutaneous melanoma: A randomised trial. *Lancet*. **358**, 866–869.
38. Kleeberg U.R., Suciu S., Bröcker E.B., Ruiter D.J., Chartier C., Liénard D., Marsden J., Schadendorf D., Eggermont A.M.M. 2004. Final results of the EORTC 18871/DKG 80-1 randomised phase III trial: rIFN-α2b versus rIFN-γ versus ISCADOR M versus observation after surgery in melanoma patients with either high-risk primary (thickness >3 mm) or regional lymph node metastasis. *Eur. J. Cancer*. **40**, 390–402.
39. Eckl Dorna J. 2012. Editorial: Natural killer cell-mediated augmentation of autoantibody production? *J. Leukoc. Biol*. **92**, 695–697.
40. O'Brien T.R. 2009. Interferon-alfa, interferon-lambda and hepatitis C. *Nat. Genet*. **41**, 1048–1050.