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A guinea pig *IFNA1* gene with antiviral activity against human influenza virus infection

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1. ABSTRACT

We previously reported a natural antisense (AS) RNA as an important modulator of human interferon-Alpha1 (*IFNA1*) mRNA levels. Here, we identified the guinea pig (*Cavia porcellus*) *IFNA1* gene to enable a proof-of-concept experiment to be performed to confirm that the AS-mRNA regulatory axis exerts *in vivo* control over innate immunity. We selected a guinea pig model system for influenza virus infection because *guinea pigs* encode a functional *M* × *1* gene, an important anti-viral effector in the type I interferon pathway. We identified 15 *guinea pig IFNA1* gene candidates upon bioinformatic analysis and selected the three candidates with the highest sequence homology to *Homo sapiens*, *Mus musculus* and *Marmota himalayana IFNA1*. The anti-viral activity of guinea pig IFN-Alpha1 protein against *influenza virus* A/Puerto Rico/8/34- or endomyocarditis virus-infection was then determined for the three gene candidates. We identified *cpIFNA1* as the candidate with the highest sequence homologies and best anti-viral effects. *cpIFNA1* will enable us to perform a proof-of-concept experiment to verify that IFN-Alpha1 AS increases *cpIFNA1* mRNA levels, resulting in inhibition of influenza virus proliferation *in vivo*.

2. INTRODUCTION

Type I interferons (IFNs) and their downstream effectors collectively limit viral proliferation and spread; therefore, IFN-Alpha-based treatments are widely used for the treatment of chronic viral infections. A large number of studies investigating IFN regulation have focused on the transcriptional activation of type I *IFN* genes and on IFN-Alpha protein function and the IFN-Alpha signal transduction cascade. However, regulation at the RNA level has received less attention (1).

During our studies on a novel *cis*-acting element that is responsible for the CRM1-dependent nuclear export of human interferon-Alpha1 (*IFNA1*) mRNA (2), we observed that deletion of this element from the

stem-loop region of the *IFNA1* mRNA selectively impaired the stability of the mRNA. This finding led us to identify IFN-Alpha1 antisense RNA (AS), a natural antisense transcript, transcribed from the opposite strand of the *IFNA1* gene that acts as an important modulator of *IFNA1* mRNA levels in both *Sendai virus*-infected human Namalwa lymphocytes and *influenza virus* A/PR/8/34 (PR/8 virus)-infected guinea pig (gp) 104C1 fetal fibroblasts by promoting *IFNA1* mRNA stability (1).

In the present study we identified the guinea pig (*Cavia porcellus*) *IFNA1* gene to enable a proof-of-concept experiment to be performed to confirm that the natural antisense transcript-mRNA regulatory axis exerts control over innate immunity *in vivo*. Mice are frequently employed as an animal model to investigate influenza A virus (IFAV) pathogenesis. However, standard laboratory mouse strains, such as BALB/c and C57BL/6, do not have a complete antiviral defense system because they carry defective alleles of the *M* × *1* gene (3, 4). The expression of *M* × *1*, which encodes the Mx GTPase, is tightly controlled by type I IFN, and is a decisive antiviral factor that controls IFAV infections in mice (5). These characteristics preclude the use of these mice for evaluating prophylactic treatment with exogenous IFN. Furthermore, the mice do not shed the virus from the respiratory tract, preventing study of proliferation profiles in the respiratory tract of individual animals without euthanasia (6).

We, therefore, employed the guinea pig, which possesses a functional *M* × *1* gene and exhibits virus shedding and is, therefore, useful for investigating the IFN response against IFAV infection (6). Although we previously published the concordant expression profiles of gp*IFNA1* mRNA/AS in 104C1 cells infected with PR/8 virus (1), we have not yet reported the identification and characterization of the *Cavia porcellus IFNA1* gene (*cpIFNA1*).

In this report, we identified fifteen *cpIFNA1* candidates upon bioinformatic analysis, all of which were localized in Scaffold 2 of the genome with comparable lengths of coding region and deduced amino acids. We selected and cloned the three *IFNA1* candidates with the highest sequence homology against *Homo sapiens*, *Mus musculus* and *Marmota himalayana IFNA1*. The anti-viral activity of guinea pig IFNA1 candidate proteins in 104C1 cells against PR/8 virus- or endomyocarditis virus (EMCV)-induced cytopathic effects was then determined. We identified *cpIFNA1* from the highest sequence homologies and the best anti-viral effects against the PR/8 and EMCV infections.

3. MATERIALS AND METHODS

3.1. Cell culture and virus propagation

Gp104C1 cells (fetal fibroblasts; ATCC CRL-1405) and MDCK (Madin-Darby canine kidney) cells (ATCC CCL-34) were maintained as previously described (1). Mouse L cells (ATCC CCL-1) were maintained in Dulbecco's minimum essential medium containing 10% heat-inactivated fetal calf serum (D10). L cells were infected with EMCV (ATCC VR-1762) at a MOI (multiplicity of infection) of 0.0.1 at 37ºC for 1 hour. The infected cells were incubated until the cytopathic effects were well advanced through 90% of the culture. The rodent-adapted PR/8 virus, *influenza virus* A/Puerto Rico/8/34 (A/PR/8/34, H1N1) (7), was propagated in the allantoic cavity of 10-day-old embryonated hen eggs and was employed to infect gp104C1 cells as previously described (1). The viral titers were measured with a plaque-forming assay using L cells for EMCV and MDCK cells for PR/8 virus.

3.2. Identification of *cpIFNA* genes

Orthologs of *IFNA* family genes in the assembled guinea pig genome (http://www.broadinstitute.org/science/projects/mammals-models/guinea-pig/guinea-pig-genome-project; released in February 2008) were searched for by comparison with existing *IFNA* family genes of *Homo sapiens*, *Mus musculus* and *Marmota himalayana* (8), using the BLAT program hosted by the UCSC Genome Browser (http://genome.ucsc.edu). All potential hits were evaluated by the presence of an open reading frame using GENETYX-MAC software (version 15; GENETYX Co., Tokyo,

Table 1. Primers used for PCR cloning of *cpIFNA1* candidate genes

Chromosome 2 - *C. porcellus IFNA1* candidate 1 gene cloning[1]		
Primer	**Strand**	**5' – 3'**
FlankF	Reverse strand 77584-77558	AGCCCAGCAGCATCAGCAGGATTACCC
FlankR	Reverse strand 76694-76739	CACAACATGTTTTATTAAAGTAACAGCATGCAGATGTTTAATGTAA
F	Reverse strand 77557-77533	cccaagcttATGGCCTGGCCATTGTCTGGACTGG
R	Reverse strand 76994-77029	gctctagaTTAGTCTTGCATCTTTCTTGCCAGTTTTTCTGATGC
Chromosome 2 - *C. porcellus IFNA1* candidate 2 gene cloning[1]		
FlankF	Plus strand180600-180637	CATTTAGGAAATCATAATCTACAAAAGTGCGTGTGGCA
FlankR	Plus strand182152-182125	CGTCCTCTGCTCCCCTCTGCAATCAAAT
F	Plus strand180878-180904	cccaagcttATGGCCTGGCCATTGACTGGATTGGTG
R	Plus strand181441-181407	gctctagaTTAGTCCCGCATCTTTCTCAACAGTTTTTCTGATG
Chromosome 2 - *C. porcellus IFNA1* candidate 3 gene cloning[1]		
FlankF	Reverse strand 114638-114611	AAGTGCATATGGCCTGTGGAGTGAAGGA
FlankR	Reverse strand 113508-113543	GAGCAAATGTAAAGAACATGTTTTATTAACTGTAAT
F	Reverse strand 114383-114357	cccaagcttATGGCCTGGCCATTGACTGGATTGGTG
R	Reverse strand 113820-113846	gctctagaTTAGTGCTGCATCTTTCTCAACAGTTC
[1]The flanking restriction sites are in lower case type		

Japan). Identification of *cpIFNA* family gene subtypes was conducted by phylogenetic analysis using ClustalW (9), available from the DNA Data Bank of Japan (http://clustalw.ddbj.nig.ac.jp).

3.3. *CpIFNA1* candidate gene expression plasmids

CpIFNA1 gene candidates 1 to 3 were amplified by polymerase chain reaction (PCR) using genomic DNA from Hartley guinea pig lung tissue as a template. The gene-specific primers used are listed in Table 1. Each amplicon was digested with *Hind*III/*Xba*I and cloned into the *Hind*III/*Xba*I sites of pSI (10) to generate pSI-*cpIFNA1* candidate 1 ~ 3 expression plasmids.

3.4. Transfection and viral protection assay

104C1 cells were subjected to magnet-assisted transfection (MATra; IBA, Goettingen, Germany) of pSI-*cpIFNA1* candidate 1, 2 or 3, and pSV-Beta-galactosidase control vector (Promega, Madison, WI, USA), as described previously (1). Culture supernatants and cell lysates were collected 48 hours after infection. The enzymatic activity of Beta-galactosidase in lysates was measured according to the manufacturer's instructions (Beta-Glo assay system; Promega) to normalize the transfection efficiency of each *cpIFNA1* expression plasmid. The culture supernatants were kept frozen at − 80°C until the viral protection assay.

For the viral protection assay, 104 C1 cells were pre-incubated for 24 h with each culture supernatant described above. Cells were then challenged with EMCV at a MOI of 0.005. The PR/8 virus challenge was conducted as previously described (1). After incubation for another 24 h (EMCV) or 48 h (PR/8), the extents to which the test culture supernatants inhibited virus-induced cytopathic events were detected by viable cell counting, as previously reported (11). Briefly, the infected cells were incubated with equal amounts of 6 mM of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl) -2H- tetrazolium salt and 0.4 mM 1-methoxy-5-methylphenazinium methylsulfate (Dojindo, Kumamoto, Japan). After addition of 1 N H_2SO_4 to stop further color development, the optical density (OD) in each culture well was measured with a microplate reader at both 450 nm and 655 nm. The net change in $OD_{450nm-655nm}$ in each well was

Table 2. List of *Cavia porcellus IFNA* family genes

SCAFFOLD	STRAND	START	END	Nucleotide length	Amino acid length
2	+	141469	142032	564	187
2	+	173473	174036	564	187
2	+	180878	181441	564	187
2	+	229967	230530	564	187
2	+	514857	515408	552	183
2	+	518755	519300	546	181
2	+	542518	543081	564	187
2	-	373323	372760	564	187
2	-	320314	319751	564	187
2	-	243481	242918	564	187
2	-	131817	131254	564	187
2	-	114383	113820	564	187
2	-	77557	76994	564	187
2	-	30926	30363	564	187
2	-	21092	20532	561	186

calculated as ($OD_{450nm-655nm}$ of the test well) - ($OD_{450nm-655nm}$ of the wells treated with the vector alone-supernatant).

3.5. Statistical analysis

Results in the Figures are representative of at least three independent experiments with triplicate samples generating similar findings. Differences presented in the Figures were analyzed using Student's *t* test.

3.6. Accession numbers

The genes employed in this study have the following accession numbers: AB671739 (*Cavia porcellus IFNA1*), AB578886 (*Homo sapiens IFNA1*), NM_010502.2. (*Mus musculus Ifna1)*, AY962656 (*Marmota himalayana IFNA1)*.

4. RESULTS

4.1. Identification and characterization of *Cavia porcellus IFNA1*

Bioinformatic analysis revealed the presence of 15 *Cavia porcellus IFNA* gene family candidates, all of which were localized in Scaffold 2 of the genome with coding region length varying from 546 nucleotides to 564 nucleotides and the number of predicted amino acids varying from 181 to 187 (Table 2; see also Figure 1A for *cpIFNA1* candidate 1 nucleotide sequence and deduced amino acid sequence). We then selected the three *cpIFNA1* candidates with the highest sequence homology against *Homo sapiens*, *Mus musculus* and *Marmota himalayana IFNA1*, with candidate 1 showing the highest homology (Table 3, top).

The deduced amino acid sequences of these *cpIFNA1* candidates showed that all of the candidate proteins harbor the conserved IFN-Alpha, Beta, Delta family signature (8, 12) in the C-terminal region (amino acids 146–164), indicating that these proteins are gpIFN-Alpha proteins (see Figure 1B for *cpIFNA1* candidate protein 1). Moreover, the candidate 1 protein showed the highest homology to both the IFN-Alpha1 proteins and the IFN signatures from the three species examined (Table 3, top and bottom, respectively). Interestingly, all of the IFN-Alpha1 candidate proteins were most homologous to the IFN-Alpha1 protein from *Marmota himalayana*, which most closely related to *Cavia porcellus*.

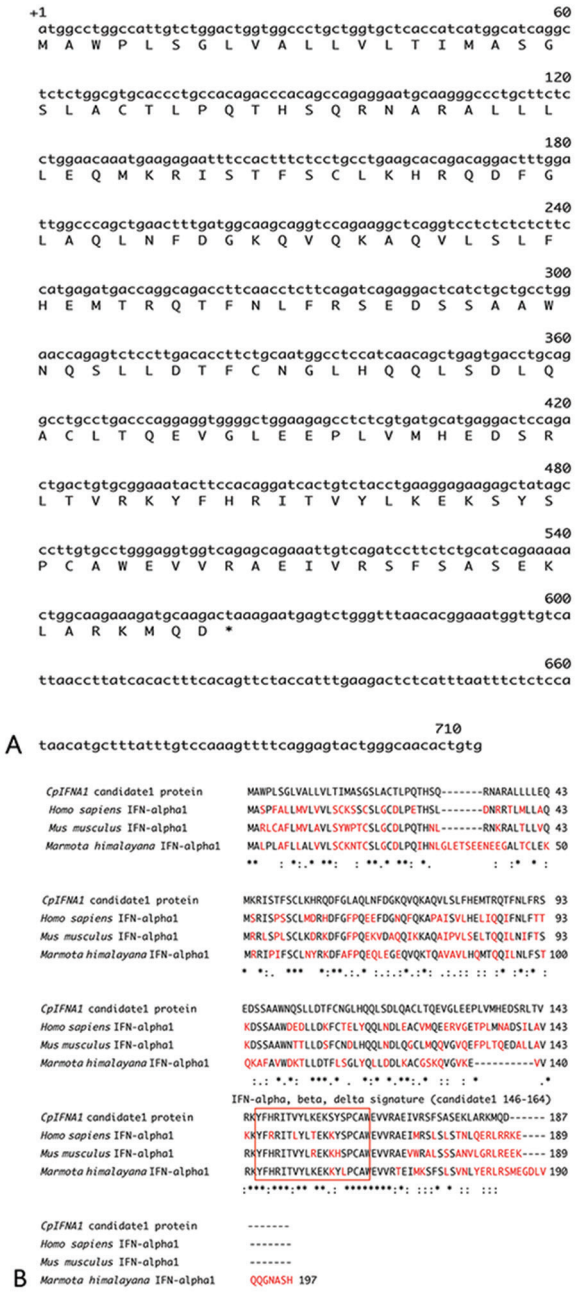

Figure 1. Characterization of the *Cavia porcellus IFNA1* candidate 1 nucleotide and deduced amino acid sequences. (A) The nucleotide sequence of *IFNA1* candidate 1 from *Cavia porcellus* is shown. The deduced amino acid sequence is indicated below the nucleotide sequence. Nucleotide residues in the coding sequence are numbered with respect to the initiation codon. * denotes stop codon. (B) Comparison of amino acid sequences for the IFN-Alpha1 proteins. Amino acid sequences of the potential IFN-Alpha1 protein encoded by *cpIFNA1* candidate 1 are aligned to *IFNA1* sequences from *Homo sapiens*, *Mus musculus* and *Marmota himalayana*. Amino acid residues are numbered with respect to the initiation methionine of *cpIFNA1* candidate 1 protein. The IFN-Alpha/Beta/Delta family signature is boxed. Gaps are shown by hyphens. "*" denotes perfect alignment. ":" and "." denote sites belonging to groups exhibiting strong and weak similarities, respectively. Strong similarity corresponds to a PAM250 MATRIX score between amino acids of greater than 0.5., whereas weak similarity corresponds to a score of 0.5. or less. The multiple amino acid sequence alignment was examined by ClustalW (9), DNA Data Bank of Japan (http://clustalw.ddbj.nig.ac.jp).

Table 3. Homology search results of the three *cpIFNA1* candidates with the highest homology against *Homo sapiens, Mus musculus* and *Marmota himalayana IFNA1* genes

CpIFNA1 candidates	Location	Percent homology of *cpIFNA1* against each *IFNA1* listed below					
		Homo sapiens IFNA1		*Mus musculus IFNA1*		*Marmota himalayana IFNA1*	
		Nucleotides	Amino acids	Nucleotides	Amino acids	Nucleotides	Amino acids
1	Scaffold 2 (77557-76994)	74.8.%	55.9.%	72.0.%	56.1.%	68.0.%	47.7.%
2	Scaffold 2 (180878-181441)	70.9.%	49.5.%	69.8.%	49.4.%	65.1.%	44.6.%
3	Scaffold 2 (114324-113820)	74.3.%	57.2.%	71.9.%	59.4.%	67.8.%	46.6.%
		Percent homology of *cpIFNA1* IFN signature against each *IFNA1* listed below					
		Homo sapiens IFNA1		*Mus musculus IFNA1*		*Marmota himalayana IFNA1*	
		Nucleotides	Amino acids	Nucleotides	Amino acids	Nucleotides	Amino acids
1	Scaffold 2 (77557-76994)	82.5.%	79.0.%	86.0.%	84.2.%	87.7.%	89.5.%
2	Scaffold 2 (180878-181441)	82.5.%	68.4.%	82.5.%	68.4.%	84.2.%	73.7.%
3	Scaffold 2 (114324-113820)	80.7.%	79.0.%	84.2.%	73.7.%	87.7.%	84.2.%

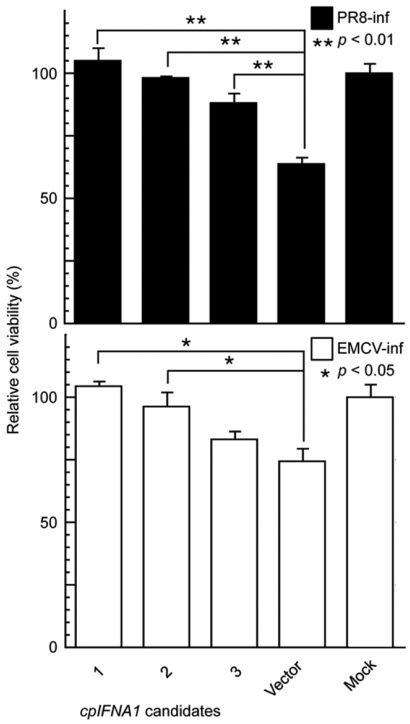

Figure 2. Antiviral assay with *cpIFNA1* candidates. 104C1 cells were transfected with pSI-*cpIFNA1* candidate 1, 2 or 3. The culture supernatants were collected and added individually to 104C1 cells. The cells were then infected with either PR/8 virus (top, solid bars) or EMCV (bottom, empty bars). The net changes in $OD_{450nm-655nm}$ were measured and used to calculate cell viability. The average net change of mock-treated and mock-infected 104C1 cells (Mock) are presented as 100% relative cell viability. Values of three independent experiments are presented as the mean ± s.e.m. of four or five samples. Vector: pSI vector-transfected and virally infected 104C1 cells.

The anti-viral activity of gpIFN-Alpha1 candidate proteins was then determined by their ability to protect guinea pig 104 C1 fetal fibroblasts against cytopathic events following PR/8 virus or EMCV infection. As shown in Figure 2, all of the *cpIFNA1* candidates showed significantly improved cell viability after infection of 104 C1 cells pretreated with culture supernatants from cells transfected with each candidate gene expression plasmid relative to the mock-treated and mock-infected 104 C1 cells, except for *cpIFNA1* candidate 3 against EMCV infection. The relative viability of PR/8 virus-infected cells varied from 88% (candidate 3) to 105% (candidate 1) compared with vector-control cells. EMCV-infected cells showed relative cell viability of 83% (candidate 3) to 104% (candidate 1), whereas the negative control cells showed 74% cell viability. Based on these biological data and the bioinformatic analysis, candidate 1 '*cpIFNA1*' showed the best antiviral effects against PR/8 and EMCV infection and had the highest homologies to both the IFN-Alpha, Beta, Delta family signature and *IFNA1* sequences.

5. DISCUSSION

We previously identified and characterized human IFN-Alpha1 AS, a natural antisense transcript and a long non-coding RNA, which plays a critical role in the post-transcriptional regulation of

IFNA1, and subsequently IFN-Alpha1 protein production (1). This regulatory function was mediated by transient duplex formation between IFN-Alpha1 AS and the mRNA, which resulted in stabilization of the *IFNA1* mRNA.

The regulatory effect of the duplex formation was verified by transfection of an antisense oligoribonucleotide (asORN), which was designed from the IFN-Alpha1 AS domain that recognizes a single-stranded target structure within a conserved secondary structure element formed in the *IFNA1* mRNA-coding region. The asORN raised the levels of *IFNA1* mRNA a few fold higher compared with levels in ncasORN-transfected cells, whereas neither the asORN nor ncasORN had any effect on IFN-Alpha1 AS expression (1).

To confirm these findings *in vivo*, we aimed to demonstrate, in a proof-of-concept experiment that asORNs designed from a model animal-derived IFN-Alpha1 AS functional domain that recognizes the *IFNA1* mRNA, increase mRNA levels, and thereby, induce antiviral effects *in vivo*. Standard laboratory mouse strains, such as BALB/c and C57BL/6, lack functional *M* × *1*, the mouse homolog of human *MX1* (4, 13); therefore, we selected the guinea pig as the model animal, which encodes this important anti-viral effector in the type I interferon pathway (6, 14).

In this work, we identified *cpIFNA1* and characterized its anti-viral function against PR/8 virus or EMCV infection. This has enabled us to set up a guinea pig model system to allow investigation of how asORNs regulate gp*IFNA1* mRNA levels, and the subsequent effects on viral titers in PR/8 virus-infected animals. In the accompanying manuscript, we report the results from the proof-of-concept experiment, showing that pulmonary-administered asORNs raise the *in vivo* levels of gp*IFNA1* mRNA, leading to inhibition of influenza virus proliferation in the animals (15).

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7. REFERENCES

1. T. Kimura, S. Jiang, M. Nishizawa, E. Yoshigai, I. Hashimoto, M. Nishikawa, T. Okumura and H. Yamada: Stabilization of human interferon-alpha1 mRNA by its antisense RNA. *Cell Mol Life Sci*, 70 (8), 1451-67 (2013)
DOI: 10.1007/s00018-012-1216-x
PMid: 23224365 PMCid: PMC3607724

2. T. Kimura, I. Hashimoto, M. Nishizawa, S. Ito and H. Yamada: Novel cis-active structures in the coding region mediate CRM1-dependent nuclear export of IFN-alpha 1 mRNA. *Med Mol Morphol*, 43 (3), 145-57 (2010)
DOI: 10.1007/s00795-010-0492-5
PMid: 20857263

3. T. M. Tumpey, K. J. Szretter, N. Van Hoeven, J. M. Katz, G. Kochs, O. Haller, A. Garcia-Sastre and P. Staeheli: The M × 1 gene protects mice against the pandemic 1918 and highly lethal human H5N1 influenza viruses. *J Virol*, 81 (19), 10818-21 (2007)
DOI: 10.1128/JVI.01116-07
PMid: 17652381 PMCid: PMC2045488

4. P. Staeheli, R. Grob, E. Meier, J. G. Sutcliffe and O. Haller: Influenza virus-susceptible mice carry Mx genes with a large deletion or a nonsense mutation. *Mol Cell Biol*, 8 (10), 4518-23 (1988) PMID: 2903437 PMCID: PMC365527
DOI: 10.1128/MCB.8.10.4518
PMid: 2903437 PMCid: PMC365527

5. O. Haller, M. Frese and G. Kochs: Mx proteins: mediators of innate resistance to RNA viruses. *Rev Sci Tech*, 17 (1), 220-30 (1998) PMID: 9638812 DOI: 10.20506/rst. 17.1.1084 PMid: 9638812

6. N. Van Hoeven, J. A. Belser, K. J. Szretter, H. Zeng, P. Staeheli, D. E. Swayne, J. M. Katz and T. M. Tumpey: Pathogenesis of 1918 pandemic and H5N1 influenza virus infections in a guinea pig model: antiviral potential of exogenous alpha interferon to reduce virus shedding. *J Virol*, 83 (7), 2851-61 (2009) DOI: 10.1128/JVI.02174-08 PMid: 19144714 PMCid: PMC2655560

7. O. Haller: Inborn resistance of ice to orthomyxoviruses. *Curr Top Microbiol Immunol*, 92, 25-52 (1981) PMID: 6171385 DOI: 10.1007/978-3-642-68069-4_3 PMid: 6171385

8. Y. Lu, B. Wang, H. Huang, Y. Tian, J. Bao, J. Dong, M. Roggendorf, M. Lu and D. Yang: The interferon-alpha gene family of Marmota himalayana, a Chinese marmot species with susceptibility to woodchuck hepatitis virus infection. *Dev Comp Immunol*, 32 (4), 445-57 (2008) DOI: 10.1016/j.dci. 2007.08.003 PMid: 17936902

9. J. D. Thompson, D. G. Higgins and T. J. Gibson: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, 22 (22), 4673-80 (1994) PMID: 7984417 PMCID: PMC308517 DOI: 10.1093/nar/22.22.4673 PMid: 7984417 PMCid: PMC308517

10. T. Kimura, I. Hashimoto, T. Nagase and J. Fujisawa: CRM1-dependent, but not ARE-mediated, nuclear export of IFN-alpha1 mRNA. *J Cell Sci*, 117(Pt 11), 2259-70 (2004) DOI: 10.1242/jcs. 01076 PMid: 15126627

11. T. Yamamoto, A. Jeevan, K. Ohishi, Y. Nojima, K. Umemori, S. Yamamoto and D. N. McMurray: A new assay system for guinea pig interferon biological activity. *J Interferon Cytokine Res*, 22 (7), 793-7 (2002) DOI: 10.1089/107999002320271387 PMid: 12184917

12. R. Doreswamy, M. Saini, D. Swaup, V. K. Singh, S. Upreti, A. Das and P. K. Gupta: Interferon alpha characterization and its comparative expression in PBMC cells of Capra hircus and Antelope cervicapra cultured in the presence of TLR9 agonist. *Molecular Biolo Intll*, 2010, 6 (2010) DOI: 10.4061/2010/573426 PMid: 22110957 PMCid: PMC3218375

13. D. Grimm, P. Staeheli, M. Hufbauer, I. Koerner, L. Martinez-Sobrido, A. Solorzano, A. Garcia-Sastre, O. Haller and G. Kochs: Replication fitness determines high virulence of influenza A virus in mice carrying functional M×1 resistance gene. *Proc Natl Acad Sci U S A*, 104 (16), 6806-11 (2007) DOI: 10.1073/pnas. 0701849104 PMid: 17426143 PMCid: PMC1871866

14. M. A. Horisberger and M. C. Gunst: Interferon-induced proteins: identification of Mx proteins in various mammalian species. *Virology*, 180 (1), 185-90 (1991) PMID: 1984648 DOI: 10.1016/0042-6822 (91) 90022-4

15. R Sakamoto, S Jiang, Y Tsukada, H Tsujimoto, T Kimura. IFN-Alpha1 antisense RNA represses human influenza A virus growth in a guinea pig system. *Front Biosci* (In Press).

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