Activation of NADPH Oxidase Complex by ISA Virus Nucleoprotein Overexpression: Alteration of SUMO-1 Protein Levels a Consequence of Cellular Oxidative Stress

Fernanda Fredericksen, Melina Villalba, Valentina Arriagada, Francisco Torres, Nicolas Maldonado, Juan K Romero and Víctor H Olavarría*

Facultad de Ciencias, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Campus Isla Teja S/N, Valdivia, Chile

***Corresponding author:** Víctor H Olavarría, Facultad de Ciencias, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Campus Isla Teja S/N, Valdivia, Chile, E-mail: volavarria@uach.cl

Received date: December 18, 2017; **Accepted date:** December 20, 2017; **Published date:** December 27, 2017

Abstract

The pathogenic mechanism of the infectious salmon anemia virus (ISAv) remains unknown. One methodological approach for solving this unknown is to understand the roles of each viral component separately. Therefore, the present study evaluated the viral nucleoprotein (NP) of ISAv to establish effects to reactive oxygen species (ROS) production and SUMOylation profile balance. Salmon head kidney-1 cells transfected with NP evidenced a strong respiratory burst activation and the genic induction of p47phox, SOD, GLURED, and Bad. Additionally, NP-transfected VERO cells showed alteration in the profile of SUMOylated proteins. Notably, pharmacological inhibition of the NADPH oxidase complex through apocynin blocked ROS production and high NP-mediated cellular ROS levels. These results suggest that the ISAv NP alone can trigger, in transfected cells, a strong production of ROS (able to

hydroxyethyl)-1-piperazineethanesulfonic acid (10 mm; pH 7.0), sodium bicarbonate (1 mg/mL), gentamicin (20 µg/mL; Gibco), and 10% fetal bovine serum (Hyclone, GE Healthcare Life Sciences, USA). Transfection kinetics considered 8, 16, and 24 h. Parallel experiments included co-stimulation with H_2O_2 (100 µM; Merck, USA) or with the pharmacological inhibitor apocynin (1 µM) [12]. Cell viability was >95% for all experimental cultures. At each sampling time-point, mRNA or protein was extracted and analysed.

NP transfection

SHK-1 and VERO cells were respectively seeded onto 12 and 6-well plates. SHK-1 cells were incubated at 16°C in 10% L-15 supplemented by 10% fetal bovine serum and 1% antibiotics (penicillin/streptomycin). VERO cells were incubated at 37°C in a humid atmosphere (5% CO_2) and in DMEM supplemented with 10% fetal calf serum and 1% antibiotic (penicillin/streptomycin). Both cell lines were incubated 24 h before transfection (≈80% confluence).

Cells were transfected with the pGFP-N1-NP plasmid (SHK-1=1000 ng cells; VERO=2500 ng cells) using Lipofectamine 2000 (Invitrogen,

Results

NP increases p47phox expression

First, NP was overexpressed in SHK-1 cells (Figure 1), and expression levels were then determined *via* RT-qPCR (Table 2) and Western blot for p47phox (Figure 2), detoxifying oxidative stress genes, and molecular markers involved in apoptosis.

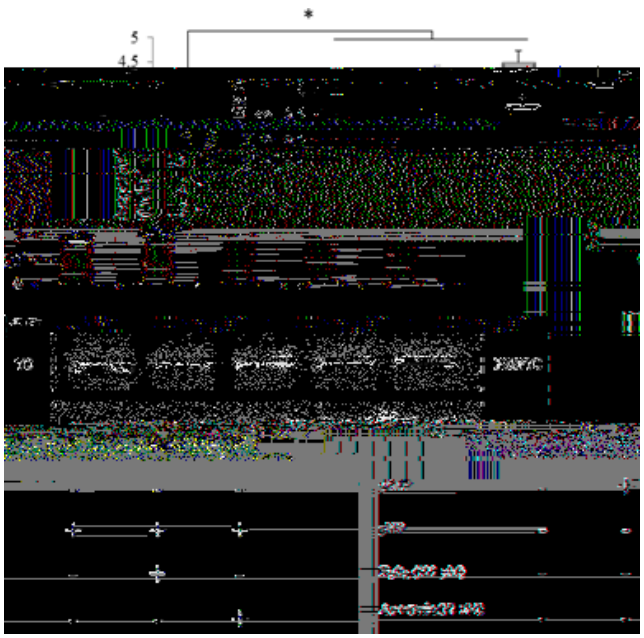

Figure 1: SUMO protein expression profles. (Top) Lane 1: VERO cell line (negative control). Lane 2: VERO cells transfected with pEGFP-N1. Lane 3: VERO cells transfected with pEGFP-N1- NP. Lane 4: VERO cells transfected with pEGFP-N1 in the presence of H_2O_2 (100 µM). Lane 5: VERO cells transfected with pEGFP-N1 in the presence of apocynin (10 µM). (Bottom) Load control using HSP70. Gene expressions were normalized against HSP70 and are shown relative to the mean expression of non-transfected cells (Line 1). Each bar represents the mean ± SE of triplicate samples. *P<0.05 versus non-transfected cells. T e results are representative of three independent experiments.

At 8 h post-transfection, p47phox expression was strongly increased and was nearly tripled at 24 h post-transfection. In the presence of H_2O_2, p47phox expression increased by 400%, but in the presence of apocynin, transcripts returned to basal levels (Table 2). Prior research revealed pro-apoptotic markers and oxygen radical detoxifers modulated during viral infection. T erefore, the efects of NP overexpression on these transcripts were assessed. At 8 h post-

of a foreign protein resulted in a 7% decrease in the SUMOylation signal level (Lane 2), a decreased that was much more drastic (47%) in NP-transfected cells (Lane 3). These findings are likely the consequence of a robust imbalance cellular ROS levels, as apparently mediated by NP. In cells transfected with NP and incubated with H_2O_2, the SUMOylation signal level slightly intensified (57%; Lane 4), while blocking of NADPH oxidase complex activity strongly increased the SUMOylation signal level (91%; Lane 5). These analyses strongly suggest that NP can modify cellular SUMOylation levels, apparently by increasing NADPH oxidase activity (Figure 3).

NP increases NADPH-mediated ROS production

To corroborate the strongly suggested capacity of NP to modulate the NADPH oxidase complex, with consequent ROS increases and SUMOylation profile changes, the effect of NP on ROS production in the absence/presence of apocynin was assessed. Oxidative stress in SHK-1 cells was detected and quantified using the CellROX reagent, a fluorogenic probe. The obtained results showed a robust fluorescent signal that was higher in cells transfected with NP than with H_2O_2 (Figure 4). In contrast, SHK-1 cells treated with apocynin had weakly decreased fluorescent signals in the cytoplasm. These results substantiate the ability of NP to activate the NADPH complex, a producer of cellular ROS.

4. Discussion

Orthomyxoviridae viruses have been extensively studied due wide reaching impact on public health. Nevertheless, essential aspects of the infection mechanisms of these viruses remain unknown. Therefore, any insights contribute a valuable contribution towards the development of tools/strategies for the containment/eradication of these pathogens, including ISAv. Considering the scarcity of cellular

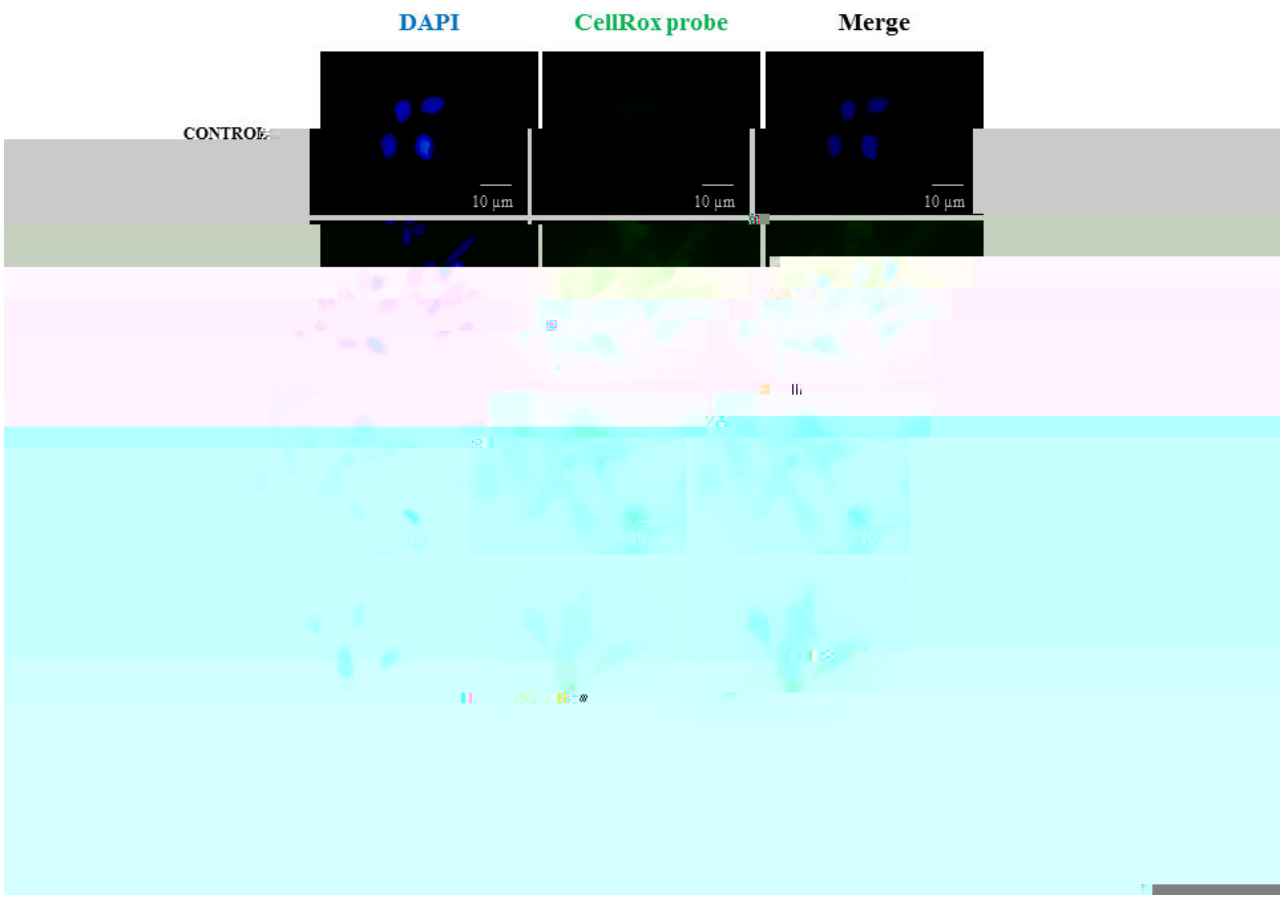

Figure 4: Suggests the imbalance in cellular SUMO activity.

Conclusions

In conclusion, ISAv infection induces an NP-mediated induction of genes encoding for NADPH oxidase components, pro-apoptotic markers, and ROS detoxifer components in SHK-1 cells. Moreover, NP was able to post-translationally modify the cellular protein profle, acting as an oxidative stressor in fsh cells.

Acknowledgments

T is work was supported by the Department of Investigation (Universidad Austral de Chile) and the Chilean National Fund for Scientifc and Technological Development (FONDECYT) [grant number 11150560].

References

1. Kibenge FS, Kibenge MJ, Groman D, McGeachy S (2006) In vivo correlates of infectious salmon anemia virus pathogenesis in fsh. J Gen Virol 87: 2645-2652.
2. Bouchard D, Keleher W, Opitz HM, Blake S, Edwards KC (1999) Isolation of infectious salmon anemia virus (ISAV) from Atlantic salmon in New Brunswick, Canada. Dis Aquat Organ 35: 131–137.
3. Kibenge FSB, Lyaku JR, Rainnie D, Hammell, KL (2000) Growth of infectious salmon anaemia virus in CHSE-214 cells and evidence for phenotypic difrences between virus strains. J Gen Virol 81: 143–150.
4. Schultz-Cherry S, Dybdahl-Sissoko N, Neumann G, Kawaoka Y, Hinshaw VS (2001) Infuenza virus ns1 protein induces apoptosis in cultured cells. J Virol 75: 7875-7881.
5. Olavarría VH, Recabarren P, Fredericksen F, Villalba M, Yáñez A (2015) ISAV infection promotes apoptosis of SHK-1 cells through a ROS/p38 MAPK/Bad signaling pathway. Mol Immunol 64: 1-8.
6. Manza

11. Takahashi M, Higuchi M, Makokha GN, Matsuki H, Yoshita M, et al. (2013) HTLV-1 Tax oncoprotein stimulates ROS production and apoptosis in T cells by interacting with USP10. Blood 122: 715-725.
12. Olavarría VH, Gallardo L, Figueroa JE, Mulero V (2010) Lipopolysaccharide primes the respiratory burst of Atlantic salmon SHK-1 cells through protein kinase C-mediated phosphorylation of p47phox. Dev Comp Immunol 34: 1242-1253.
13. Olavarría VH, Figueroa JE, Mulero V (2012) Prolactin-induced activation of phagocyte NADPH oxidase in the teleost fsh gilthead seabream involves the phosphorylation of p47phox by protein kinase C. Dev Comp Immunol 36: 216-221.
14. Vlahos R, Stambas J, Bozinovski S, Broughton BR, Drummond GR, et al. (2011) Inhibition of Nox2 oxidase activity ameliorates infuenza A virus-induced lung infammation. PLoS Pathog 7: 1.
15. Compans RW, Content J, Duesberg PH (1972) Structure of the ribonucleoprotein of infuenza virus. J Virol 10: 795–800.
16. Ortega J, Martin-Benito J, Zurcher T (2000) Ultrastructural and functional analyses of recombinant infuenza virus ribonucleoproteins suggest dimerization of nucleoprotein during virus amplifcation. J Virol 74: 156–163.
17. Prokudina EN, Semenova NP, Chumakov VM (2005) Stability of intracellular infuenza virus nucleocapsid protein oligomers. Arch Virol 150: 833–839.
18. Chou YY, Vafabakhsh R, Doganay S (2012) One infuenza virus particle packages eight unique viral RNAs as shown by FISH analysis. Proc Natl Acad Sci 109: 9101–9106.
19. Okura T, Gong L, Kamitani T, Wada T, Okura I, et al. (1996) Protection against Fas/APO-1-and tumor necrosis factor-mediated cell death by a novel protein, sentrin. J Immunol 157: 4277–4281.
20. Bossis G, Melchior F (2006) SUMO: regulating the regulator. Cell Div 1: 13.
21. Wang L, Banerjee S (2004) Diferential PIAS3 expression in human malignancy. Oncol Reports 11: 1319–1324.
22. Wang CY, Podolsky R, She JX (2006) Genetic and functional evidence supporting SUMO4 as a type 1 diabetes susceptibility gene. Ann N Y Acad Sci 1079: 257–267.
23. Lee HS, Lim YS, Park EM, Baek SH, Hwang SB (2014) SUMOylation of nonstructural 5A protein regulates hepatitis C virus replication. J Viral Hepat 21: 1.
24. Everett RD, Boutell C, Pheasant K, Cuchet-Lourenço D, Orr A (2014) Sequences related to SUMO interaction motifs in herpes simplex virus 1 protein ICP0 act cooperatively to stimulate virus infection. J Virol 88: 2763-2774.
25. Scherer M, Reuter N, Wagenknecht N, Otto V, Sticht H, et al. (2013) Small ubiquitin-related modifer