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Original Article

A proteomic study of the differential protein expression in MDBK cells after bovine herpesvirus type 1 infection (BHV-1) strain treatment

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Abstract: Different BHV-1 strains, such as the virulent IBRV LN01/08 strains and the attenuated vaccine strain IBRV LNM, produces different clinical immune responses; however, the study of the differential protein expression in Madin-Darby bovine kidney (MDBK) cells after BHV-1-infection still remains unclear. Here, we applied a comparative proteomic strategy, based on 2D and MALDI-TOF/MS platforms, to examine the differential expression of proteins in MDBK cells that were treated and not treated with virulent IBRV LN01/08 and attenuated IBRV LNM strains. A total of eight differential proteins, including pyruvate kinase, heat shock protein (HSP) 90 (HSP90AA1 and HSP90AB1), annexin A, albumin (ALB), scinderin (SCIN), tubulin (alpha 1a) and vimentin (VIM), were identified. Among these proteins, pyruvate kinase, and HSP90 (HSP90AB1), tubulin and vimentin were identified in the virulent IBRV LN01/08 strain group, but were not identified in the attenuated IBRV LNM group. These results play an important role in tumor formation and development, cell migration, tumor cell line apoptosis, cell invasion and viral infection. The HSP90 (HSP90AA1) protein was identified in the control group and the attenuated IBRV LNM-infected group. Most studies have shown that HSP90 proteins were more of a cancer gene target, and inhibiting its function would result to oncogene degradation during cancer treatment. On the other hand, ALB is associated to cell differentiation, apoptosis, necrosis, cell death, viral infection, autophagy, interstitial tissue inflammation, and cell survival. These results provide a theoretical basis for the systematic understanding of BHV-1-infection mechanisms and BHV-1-induced immune responses.

Keywords: BHV-1, MDBK, comparative proteome

Introduction

Infectious bovine rhinotracheitis (IBR) is a well-known contagious and infectious disease, worldwide. The pathogen is also known as bovine herpesvirus type 1 infection (BHV-1), which belongs to the genus Varicellovirus in the subfamily of Alphaherpesvirinae, under the family of Herpesviridae [1, 2]. Herpesviruses are large, enveloped, double-stranded DNA viruses [3], having a 135-140kbp size [4, 5] and codes for 70 proteins. The BHV-1 genome is a huge, complex structure; and research on the structure and function of BHV-1 remains to be discovered [6]. BHV-1 affects cattle, goats, sheep, buffaloes, and camelids; and also infects pronghorn antelopes, wildebeest, mink, and ferrets [7-9] Porter et al., 1975. The virus hides in the trigeminal ganglion, sheds from latency following reactivation, and transmission occurs by contact with mucosal droplets from the infected cattle [10]. BHV-1 is one of the agents that cause Bovine Respiratory Disease Complex (BRDC) [11, 12]. Immunity to BHV-1 commonly occurs due to either natural infection or vaccination [13, 14]. Immunity involves both cellular and humoral response to various glycoproteins [15]. The effective control of IBR can only be possible with an increased understanding of both the respiratory and immune systems of the cow; as well as host, pathogen, and environmental interactions.

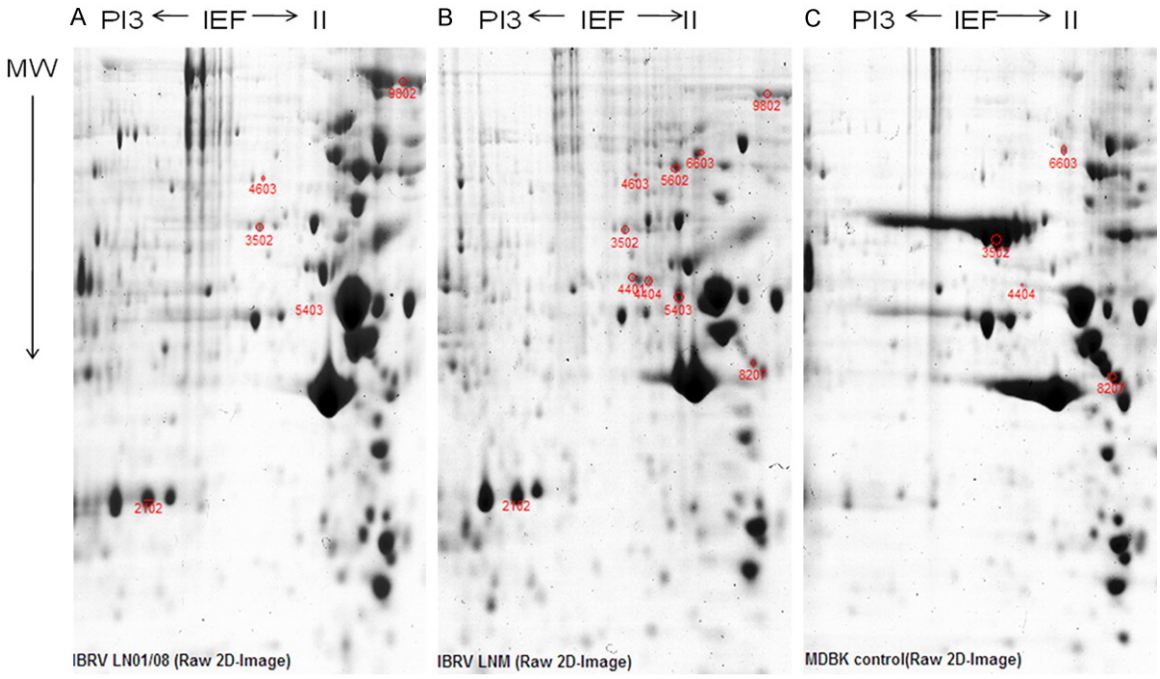

Figure 1. Proteomic analysis of MDBK cells from the control and experimental groups (36 hours of post-exposure) using 2-DE gels. Proteins on the 2-DE gels of the experimental group (A, B) and control group (C) were visualized using Coomassie® Blue Staining.

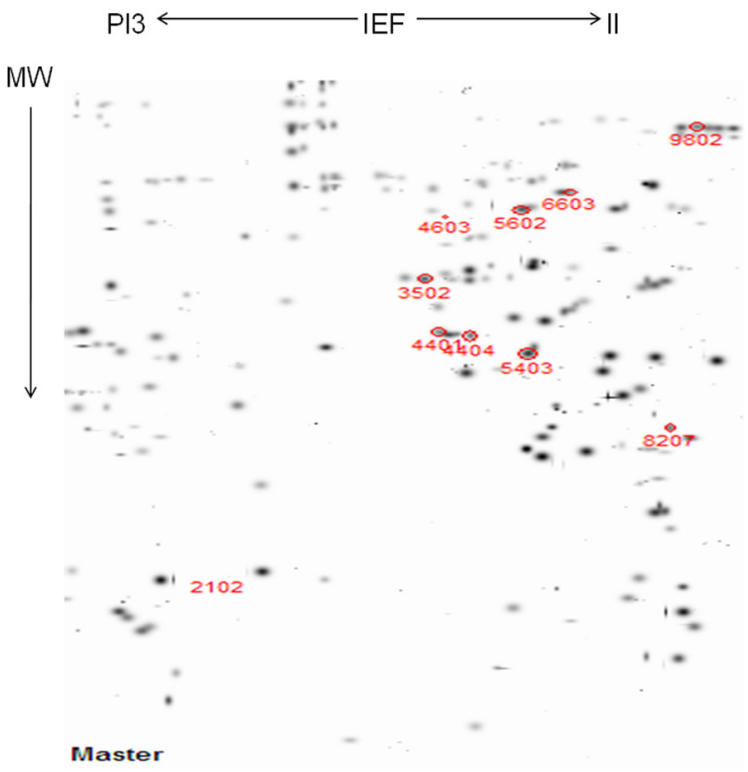

Figure 2. The differential expression of proteins, which were analyzed using PDQuest. PDQuest software (BIO-RAD) was used to analyze the scanned 2-DE gel image. Spots representing different significant protein expressions were selected (**Table 1**) for further mass spectrometric analysis.

In studying attenuated infectious bovine rhinotracheitis vaccines, it was noted, -during screening-that the virulent strain IBRV LN01/08 was domesticated in the MBDK host cell, and the attenuated virus strain, called IBRV LNM, retained its immunogenicity, but its virulence was significantly reduced. After inoculating cattle, the two strains exhibited different immune responses. The virulent IBRV LN01/08 strain showed significant infectious bovine rhinotracheitis clinical symptoms, such as fever, running nose, nasal mucosal swelling, ulceration and conjunctivitis. However, the attenuated strain IBRV LNM showed an increased virulent protective resistance. In sequencing the virulence and attenuated strain genes with TK, U21, U24 and U4 genes [16, 17], differences between genome virulence key sites were not found.

Table 1. The numerical values of protein spots with different protein expressions were analyzed using PDQuest software. Standard spot (SSP) numbers were assigned to each protein spot by PDQuest software and each SSP number uniquely identifies one protein. The "-" means no spot in the 2-DE gel image. Groups BRV LN01/08 and BRV LNM are the experimental groups; Group C is the Control group

SSP	Group IBRV LN01/08	Group BRV LNM	Group C
2102	-	486.2	24.5
3502	16649.1	6070.7	8224.3
4401	-	-	6750.4
4404	2029.3	-	6288.3
4603	-	1512.5	651.1
5403	-	110.5	12557.3
5602	-	-	9035.7
6603	5794.8	-	3870.8
9802	9258.4	-	3626.9
8207	-	6341.5	6519.9

Since no difference in key sites were found by sequencing the main virulence gene, would virulence change due to certain protein structure, function, location or change in expression? This research analyzes the different pathogenic strains of IBRV LN01/08, which may lead to MDBK cell proteome changes through infected MDBK host cells, provide better insights in understanding the pathogenic molecular mechanisms of IBRV, and establish a theoretical basis for different pathogenic strains.

Results and discussion

2-DE and mass spectrometry analysis

In the following 2-DE (**Figure 1**) and PDQuest software analysis (**Figure 2**; **Table 1**) results, 10 protein spots were selected and enzymatically hydrolyzed. MALDI-TOF/MS identification was performed on the 10 proteins and 8 were successfully identified: annexin A2 (SSP2102), serum albumin (SSP3502), pyruvate kinase (SSP4401 and SSP4402), adseverin (SSP4604), tubulin (SSP5402), heat shock protein (SSP5602; SSP6603), vimentin (SSP8207), and an uncharacterized protein (SSP9802) (**Table 2**).

BHV-1 is a double-stranded DNA virus, having a size of 135-140 kbp [18, 19], and codes for 70 proteins, wherein, most of their structures and functions have already been known [20]. A typical herpesvirus virion consist of a core that contain a linear double-stranded DNA and an icosadaltahedral capsid of about 100 nm in diameter-containing 162 capsomeres [21]. In the BHV-1 genome, a total of 73 open reading frame (ORF) codes for proteins have been identified [22]. Even though the whole IBRV genome has already been sequenced, many gene functions are still unknown. The IBRV LN01/08 strain and the attenuated vaccine strain, IBRV LNM, completely produce different clinical immune responses. A total of eight differential proteins, including pyruvate kinase, heat shock protein 90 (HSP90AA1 and HSP90AB1), annexin A, albumin, scinderin, tubulinand, and vimentin, were identified based on the 2D and MALDI-TOF/MS platforms-which are immune responses to receptor and cytoskeletal proteins. Pyruvate kinase, heat shock protein 90 (HSP90AB1), tubulin, and vimentin proteins were identified in the virulent IBRV LN01/08 strain group, but were not identified from the attenuated IBRV LNM group; which plays an important role in tumor formation and development, cell migration, tumor cell line apoptosis, cell invasion and viral infection [23-26]. Tubulin and pyruvate kinase proteins are used as anti-cancer drug treatments for targeted sites [27-29]. PKM is connected with pulmonary fibrosis [30], which is in agreement with the clinical response of the IBRV LN01/08 strain after inoculation. HSP90 (HSP90AA1) protein was identified in the control group and the attenuated IBRV LNM-infected group, and most studies have shown that the HSP90 protein was able to target various cancer genes and its inhibiting function can result to oncogene degradation for cancer treatment [31, 32]. HSP90AA1 is a molecular chaperone involved in cell regulation and maintains the conformation and function of cell proteins, in order to maintain cell survival under stimulation [33, 34]. These proteins have direct effects on each other. A certain protein function is usually achieved by interacting with other proteins. Further tests are needed to clarify which of the above identified proteins is associated with the pathogenesis of BHV-1. Although ALB had a lower expression level in the experimental groups, it is still associated with cell differentiation, apoptosis, necrosis, cell death, viral infection, autophagy, interstitial tissue inflammation and cell survival [35-40],

Table 2. MALDI-TOF/MS identification of differentially expressed proteins. Selected protein spots were obtained using EXQuest Spot Cutter (BIO-RAD); the proteins were enzymatically hydrolyzed and identified using MALDI-TOF/MS. Proteins with a score of 100, such as 100% homology, were included. ↑higher expression; ↓lower expression

SSP	ID	NAME	Protein MW	PI	Difference
2102	IPI00706002	ANXA2 Annexin A2	38872.9	6.92	↑
3502	IPI01028455	ALB Serum albumin	71244.2	5.82	↓
4401	IPI00839408	PKM2 Pyruvate kinase	58482.3	7.96	↑
4404	IPI00839408	PKM2 Pyruvate kinase	58482.3	7.96	↑
4603	IPI00706141	SCIN Adseverin	80983.7	5.67	↑
5403	IPI00700865	TUBA1A Tubulin	50803.9	4.94	↑
5602	IPI00709435	HSP90AB1 Heat shock protein HSP 90-beta	83543.2	4.97	↑
6603	IPI00699622	HSP90AA1 Heat shock protein HSP 90-alpha	85076.8	4.93	↓
9802	IPI00726312	DYNC1H1 Uncharacterized protein	534455.8	6.01	↓
8207	IPI00689228	VIM Vimentin	53752.1	5.06	↑

Table 3. Part of the IPA analysis results for the diseases or functional annotation of the eight differentially expressed proteins in IBRV LN01/08 and IBRV LNM treated cells

Diseases or Functional Annotation	*p*-Value	Molecules
Cholangiocarcinoma	3.38E-12	ALB, ANXA2, HSP90AA1, HSP90AB1, PKM
Metastatic occult primary head and neck cancer	4.58E-08	HSP90AA1, HSP90AB1, TUBA1A
Metastatic endometrial carcinoma	6.84E-08	HSP90AA1, HSP90AB1, TUBA1A
Unresectable nasopharyngeal cancer	7.73E-08	HSP90AA1, HSP90AB1, TUBA1A
Metastatic non-small cell lung cancer	3.93E-07	HSP90AA1, HSP90AB1, TUBA1A
Necrosis	5.87E-06	ALB, ANXA2, HSP90AA1, HSP90AB1, PKM, SCIN, TUBA1A
Apoptosis	6.62E-06	ALB, ANXA2, HSP90AA1, HSP90AB1, PKM, SCIN, TUBA1A
Tumor cell line death	7.43E-06	ALB, ANXA2, HSP90AB1, PKM, SCIN, TUBA1A
Brain tumor development	7.53E-06	ANXA2, PKM
Cells migration	2.55E-05	ALB, ANXA2, HSP90AA1, HSP90AB1, PKM, TUBA1A
Autoimmune pancreatitis	2.88E-05	ALB, PKM
Tumor cell line apoptosis	5.90E-05	ALB, ANXA2, HSP90AB1, SCIN, TUBA1A
Cell proliferation	6.62E-05	ALB, ANXA2, HSP90AA1, HSP90AB1, PKM, SCIN, TUBA1A
Astrocyte cell death	6.76E-05	ALB, HSP90AA1
Basal ganglia disorder	2.24E-04	ANXA2, HSP90AA1, PKM, TUBA1A
Cell invasion	2.54E-04	ANXA2, HSP90AA1, HSP90AB1, PKM
Viral infection	3.19E-04	ALB, ANXA2, HSP90AA1, HSP90AB1, TUBA1A
Tumor cell death	3.98E-04	ALB, ANXA2, HSP90AB1
Antigen presentation	4.51E-04	HSP90AA1, HSP90AB1
Pancreatic cancer cell line autophagy	1.46E-03	ALB
Kidney cell line proliferation	1.79E-03	HSP90AA1, HSP90AB1
Virus assembly	1.82E-03	ANXA2
Interstitial tissue inflammation	2.55E-03	ALB
Tumor cell apoptosis	5.83E-03	ALB, ANXA2
Virus attachment	5.83E-03	ANXA2
Macrophage infection	8.37E-03	ANXA2
Systemic inflammatory response syndrome	9.82E-03	ALB
Acute respiratory distress syndrome	1.42E-02	ALB
Dendritic cell response to immunization	1.49E-02	HSP90AA1
Long-term memory	1.99E-02	PKM
RNA virus replication	2.03E-02	HSP90AA1, HSP90AB1
Cell survival	2.13E-02	ALB, HSP90AB1, PKM
Macrophage proliferation	2.14E-02	ALB
Cellular homeostasis	2.33E-02	ALB, ANXA2, HSP90AA1

Ulcerative colitis	3.77E-02	ALB
Tumor growth	4.36E-02	ANXA2, PKM
Blood cell differentiation	4.45E-02	HSP90AA1, SCIN
Delayed hypersensitivity reaction	4.90E-02	ALB

as shown in **Table 3**. This research provides data for supporting the relative study of BHV-1-infected pathogenic mechanisms and BHV-1-induced immune responses.

Infectious bovine rhinotracheitis (IBR) is a disease that is typically characterized by upper respiratory infection, caused by bovine herpesvirus-1 or BHV-1 [41]. Prevalent worldwide, IBRV antibodies are detected in almost all cattle. IBR affects milk production and bull work capacity, causing huge economic losses to the cattle industry every year. Persistent and latent infections in nature are the main causes of the disease [42, 43]. Vaccines are still the main methods of prevention and control [44, 45]; however, existing vaccines are still unsatisfactory. Further, attenuated vaccines may also produce recessive infections and virulence returns. Due to latent herpes virus infections and the genetic characteristics of the recombinant [46], vaccine strains and wild strains may be infected at the same time and genetic recombination may occur, after gene-deleted vaccines are administered to the animal's body. This research focuses on the virulence determining factors and proteome differences; by using protein expression differences produced after normal MDBK cell inoculation on IBRV mutant strains, cultured in vitro with two-dimentional gel electrophoresis; the research also explores virulence related proteins, analyzes the impact of avirulent and virulent strains on cell expressing proteins, and seeks for disease related genes or proteins.

Materials and methods

Virus and cells

The virulent IBRV LN01/08 strain and the attenuated vaccine strain IBRV LNM were isolated, passaged, and preserved in our laboratory; and the viruses were propagated in the MDBK cell using the Dulbecco's Modified Eagle Medium (DMEM, HyClone), containing antibiotics (100,000 IU/L of Benzylpenicillin and 100 mg/L of streptomycin) with 10% horse serum.

Virus inoculation and sample preparation

When MDBK cell density exceeded 95% confluence, the cells were washed three times with a phosphate buffered saline (PBS) solution and 2 × 106 MDBK cells were then inoculated with 104TCID50 (50% tissue culture infective dose) of either the IBRV LN01/08 strain or the IBRV LNM strain. MDBK cells, without the virus, were used as the control. After one hour of infection, the culture medium was discarded and replaced with the Dulbecco's Modified Eagle Medium, containing 2% horse serum. Cell culture was continued and periodic observations of the cytopathic effects were performed. The culture medium was discarded 36 hours after inoculation. After gently washing the culture plate three times with pre-cooled sterilized PBS (pH 7.2-7.4) solution at 4°C, the PBS solution was discarded and the culture plate was placed on ice. A Lysis buffer (pH 8.5) containing 7 M urea, 2 M thiourea, 65 mM Tris, 2% dithiotreitol (DTT), 4% 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate (CHAPS), 0.2% IPG buffer (GE Healthcare, Munich, Germany), and 0.1% v/v protease inhibitor mixture was added into each well. All cells were scraped into a 1.5 mL centrifuge tube and homogenized with a Dounce homogenizer. The cells were disrupted by sonication (80 W, five times for a 10 s interval each time at 15 s intervals). All procedures were performed on ice. The mixture was centrifuged at 15,000 g for 45 minutes at 4°C. The protein content of the supernatants were determined by Bradford assays (Bio-Rad, USA). Aliquot samples were stored at -80°C, until used for proteomic analysis.

Protein sample preparation and 2-DE

Cell-culture material samples from the control and experimental groups (36 hours of post-exposure) were crushed by liquid nitrogen and suspended in a lysis buffer (9.5 M urea, 4% CHAPS, 65 mM DTT, and 0.2% carrier ampholyte), which contained a protease inhibitor cocktail (Roche, Mannheim, Germany). The suspension was homogenized, sonicated on ice and centrifuged at 14,000 rpm for 1 hour at

4°C. The supernatant was collected and the protein concentration was determined using a Protein Assay kit (Bio-Rad, Richmond, VA, USA). Protein aliquots equivalent to 100 mg were stored at -80°C for future use. 2-DE (Bio-Rad) was used to separate proteins, as described by De-la-Pena C et al. [47]. The 2-DE gels were scanned using a GS-710 imaging densitometer, and the digitized images were analyzed by the PDQuest software (Bio-Rad).

Mass spectrometry and peptide mass fingerprint

Protein spots were cut from the gels, destained, and washed. The spots were kept in 0.2 M $NH4HCO_3$ for 20 minutes, and lyophilized. Each spot was digested overnight with 12.5 ng/mL trypsin in 0.1 M $NH4HCO_3$, and the digested proteins were extracted three times with a 50% ACN and 0.1% TFA solution. Mass spectra data were acquired by an AutoFlex MALDI-TOF/TOF mass spectrometer with LIFT technology (Bruker Daltonics, Bremen, Germany). MS/MS data were acquired with a N2 laser at a 25-Hz sampling rate [48]. PMF and MS data were combined using FlexAnalysis and the combined data set was submitted to MASCOT [49, 50] for protein identification. The MASCOT result was a Probability-based Mowse Score [51], which was expressed as -10 × log (p), where p is the probability. Protein scores greater than 63 are considered statistically significant ($P < 0.05$). The above process was repeated three times. The criteria for selecting protein spots are: (i) spots showing greater than two-fold expression changes between the control and experimental groups; (ii) reproducible spots which were detected in all three replicated experiments; (iii) spots were successfully identified by MALDI-TOF analysis.

Conclusions

The present study identified the differentially expressed proteins in MDBK host cells. Through proteomic analysis, we found that eight proteins, including pyruvate kinase, heat shock protein 90 (HSP90AA1 and HSP90AB1), annexin A, albumin (ALB), scinderin (SCIN), tubulin (alpha 1a) and vimentin (VIM), occurred at different MDBK cell levels -- after IBRV LN01/08 and IBRV LNM infection. These key proteins may be related to pathogenic mechanisms and BHV-1-induced immune responses. Further studies must focus in clarifying the mechanisms and roles for these proteins in virulent IBRV LN01/08 and attenuated vaccine strains, during the immune response process.

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Disclosure of conflict of interest

None.

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References

[1] Muylkens B, Thiry J, Kirten P, Schynts F and Thiry E. Bovine herpesvirus infection and infectious bovine rhinotracheitis. Vet Res 2007; 38: 181-209.

[2] Nandi S, Kumar M, Manohar M and Chauhan RS. Bovine herpes virus infections in cattle. Anim Health Res Rev 2009; 10: 85-98.

[3] Harrison SC. Principles of virus structure. Chapter 3. In: Knipe DM, Howley PM, editors. Fields' virology. 4th edition. Volume 1. Philadelphia: Lippincott Williams & Wilkins; 2011. pp. 53-85.

[4] Mayfield JE, Good PJ, Vanoort HJ, Campbell AR and Reed DE. Cloning and cleavage site mapping of DNA from bovine herpesvirus 1 (cooper strain). J Virol 1983; 47: 259-264.

[5] Wyler R, Engles M and Schwyzer M. Infectious bovine rhinotracheitis/vulvovaginitis (BHV-1). In: Wittmann G, editor. Herpesvirus diseases of cattle, horses and pigs. Developments In Veterinary Virology. Boston: Kluwer Academic Publishers; 1989. pp. 1-172.

[6] Engels M, Giuliani C, Wild P and Beck. The genome of bovine herpesvirus 1 (BHV-1) strains exhibiting a neuropathogenic potential compared to known BHV-1 strains by restriction site mapping and cross-hybridization. J Virus Res 1986; 6: 57-73.

[7] Whetstone CA and Evermann JF. Characterization of bovine herpes viruses isolated from six

sheep and four goats by restriction endonuc lease analysis and radio immunoprecipitation. Am J Vet Res 1988; 49: 781-785.
[8] Metzler AE, Ossent P, Guscetti F, Rubel A and Lang EM. Serological evidence of herpesvirus infection in captive Asian elephants (Elephas maximus). J Wildl Dis 1990; 26: 41-49.
[9] Bhat MN, Manickam R and Kumanan K. Serological evidence of bovine herpesviruses 1 and 2 in Asian elephants. J Wildl Dis 1997; 33: 919-920.
[10] Kahrs RF. Infectious bovine rhinotracheitis and infections pustular vulvovaginitis. Viral Diseases of Cattle. 2nd edition. Ames. IA: Iowa State University Press; 2001. pp. 159-170.
[11] Winkler MT, Doster A and Jones C. Bovine herpesvirus 1 can infect CD4(t) T lymphocytes and induce programmed cell death during acute infection of cattle. J Virol 1999; 73: 8657-8668.
[12] Lovato L, Inman M, Henderson G, Doster A and Jones C. Infection of cattle with a bovine herpesvirus 1 strain that contains a mutation in the latency-related gene leads to increased apoptosis in trigeminal ganglia during the transition from acute infection to latency. J Virol 2003; 77: 4848-4857.
[13] Denis M, Slaoui M, Keil G, Babiuk LA, Ernst E, Pastoret PP and Thiry E. Identification of different target glycoproteins for bovine herpesvirus-specific cytotoxic T lymphocytes depending on the method of in vitro stimulation.Immunology 1993; 78: 7-13.
[14] Tikoo SK, Campos M and Babiuk LA. Bovine herpesvirus 1 (BHV-1): biology, pathogenesis, and control. Adv Virus Res 1995; 45: 191-223.
[15] Babiuk LA, van Drunen Littel-van den Hurk and Tikoo SK. Immunology of bovine herpesvirus 1 infection. Vet Microbiol 1996; 53: 31-42.
[16] Jones C, Geiser V and Henderson G. Functional analysis of bovine herpesvirus 1 (BHV-1) genes expressed during latency. J Vet Micro 1996; 113: 199-210.
[17] Muylkens B, Thiry J, Kirten P, Schynts F and Thiry E. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Vet Res 2007; 38: 181-209.
[18] Mayfield JE, Good PJ, Vanoort HJ, Campbell AR and Reed DE. Cloning and cleavage site mapping of DNA frombovine herpesvirus 1 (cooper strain). J Virol 1983; 47: 259-264.
[19] Wyler R, Engles M and Schwyzer M. Infectious bovine rhinotracheitis/vulvovaginitis (BHV-1). In: Wittmann G, editor. Herpesvirus diseases of cattle, horses and pigs, Developments In Veterinary Virology. Boston: Kluwer Academic Publishers; 1989. pp. 1-172.
[20] Mayfield JE, Good PJ, Vanoort HJ, Campbell AR and Reed DE. Cloning and cleavage site mapping of DNA from bovine herpesvirus 1 (cooper strain). J Virol 1983; 47: 259-264.
[21] Roizman B, Knipe DM and Whitley RJ. Herpes Simplex Virus. In: David M, Knipe DM, Howley PM, editors. Fields'virology. 5th edition. Volume 2. Philadelphia: Lippincott Williams & Wilkins.
[22] Liang X, Chow B, Raggo C and Babiuk LA. Bovine herpesvirus1 UL49.5 homolog gene encodes a novel viral envelope protein that forms a disulfide-linked complex with a second virion structural protein. J Virol 1996; 70: 1448-1454.
[23] Ernst JT, Neubert T, Liu M, Sperry S, Zuccola H, Turnbull A, Fleck B, Kargo W, Woody L, Chiang P, Tran D, Chen W, Snyder P, Alcacio T, Nezami A, Reynolds J, Alvi K, Goulet L and Stamos D. Identification of novel HSP90 α/β isoform selective inhibitors using structure-based drug design. demonstration of potential utility in treating CNS disorders such as Huntington's disease. J Med Chem 2014; 57: 3382-400
[24] Coskunpinar E, Akkaya N, Yildiz P, Oltulu YM, Aynaci E, Isbir T and Yaylim I. The significance of HSP90AA1, HSP90AB1 and HSP90B1 gene polymorphisms in a Turkish population with non-small cell lung cancer. Anticancer Res 2014; 34: 753-757.
[25] Haase MG, Geyer P, Fitze G, Baretton GB and J Histochem Cytochem. Down-regulation of heat shock protein HSP90ab1 in radiation-damaged lung cells other than mast cells. J Histochem Cytochem 2014; 62: 355-368.
[26] Huang W, Ye M, Zhang LR, Wu QD, Zhang M, Xu JH and Zheng W. FW-04-806 inhibits proliferation and induces apoptosis in human breast cancer cells by binding to N-terminus of Hsp90 and disrupting Hsp90-Cdc37 complex formation. Mol Cancer 2014; 13: 150.
[27] Pox CP. Controversies in Colorectal Cancer Screening. Digestion 2014; 89: 274-281.
[28] Husain M and Cheung CY. Histone deacetylase 6 inhibits influenza A virus release by down-regulating the trafficking of viral components to the plasma membrane via its substrate acetylated microtubules. J Virol 2014; 88: 11229-39.
[29] La Regina G, Bai R, Coluccia A, Famiglini V, Pelliccia S, Passacantilli S, Mazzoccoli C, Ruggieri V, Sisinni L, Bolognesi A, Rensen WM, Miele A, Nalli M, Alfonsi R, Di Marcotullio L, Gulino A, Brancale A, Novellino E, Dondio GM, Vultaggio S, Varasi M, Mercurio C, Hamel E, Lavia P and Silvestri R. JNew Pyrrole Derivatives with Potent Tubulin Polymerization Inhibiting Activity as Anticancer Agents including Hedgehog-dependent Cancer. Med Chem 2014; 57: 6531-52.

[30] Lee IK. The role of pyruvate dehydrogenase kinase in diabetes and obesity. Diabetes Metab J 2014; 38: 181-186.
[31] Okayama S, Kopelovich L, Balmus G, Weiss RS, Herbert BS, Dannenberg AJ and Subbaramaiah K. p53 protein regulates Hsp90ATPase activity and thereby Wnt signaling by modulating Aha1 expression. J Biol Chem 2014; 289: 6513-6525.32.
[32] Acquaviva J, He S, Zhang C, Jimenez JP, Nagai M, Sang J, Sequeira M, Smith DL, Ogawa LS, Inoue T, Tatsuta N, Knowles MA, Bates RC and Proia DA. FGFR3 Translocations in Bladder Cancer: Differential Sensitivity to HSP90 Inhibition Based on Drug Metabolism. Mol Cancer Res 2014; 12: 1042-1054.
[33] Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 2010; 10: 537-549
[34] Yang PH, Zhang L, Zhang YJ, Zhang J and Xu WF. HDAC6: physiological function and its selective inhibitors for cancer treatment. Drug Discov Ther 2013; 7: 233-242.
[35] Horio M, Imai E, Yasuda Y, Watanabe T, Yokoyama H, Makino H and Matsuo S. Serum albumin, but not glycated albumin was a potent factor affecting the performance of GFR equation based on serum creatinine. Clin Exp Nephrol 2014; 19: 284-92.
[36] Dueñas F, Becerra V, Cortes Y, Vidal S, Sáenz L, Palomino J, De Los Reyes M amd Peralta OA Hepatogenic and neurogenic differentiation of bone marrow mesenchymal stem cells from abattoir-derived bovine fetuses. BMC Vet Res 2014; 10: 154.
[37] Moore AR, Allender MC and MacNeill AL. Effects of ranavirus infection of red-eared sliders (Trachemys scripta elegans) on plasma proteins. J Zoo Wildl Med 2014; 45: 298-305.
[38] Lu LR, Liu J, Xu Z, Zhang GL, Li DC and Lin CS. Expression and clinical significance of myeloid derived suppressor cells in chronic hepatitis B patients. Asian Pac J Cancer Prev 2014; 15: 4367-72.
[39] Wang D, Song C, Shen HW and Barabási AL. Science communication. Response to Comment on "Quantifying long-term scientific impact". Sci 2014; 345: 149.
[40] Alizadeh E, Zarghami N, Eslaminejad MB, Akbarzadeh A, Barzegar A and Mohammadi SA. The effect of dimethyl sulfoxide on hepatic differentiation of mesenchymal stem cells. Artif Cells Nanomed Biotechnol 2014; 30: 1-8.
[41] Mars MH, de Jong MC, van Maanen C, Hage JJ and van Oirschot JT. Airborne transmission of bovine herpesvirus 1 in calves under field conditions. J Vet Microbiol 2014; 76: 1-13.
[42] Abril C, Engels M and Liman A. Both viral and host factors contribute to neurovirulence of bovine herpesviruses 1 and 5 in interferon receptor-deficient mice. J Virology 2004; 78: 3644-3653.
[43] Clinton J. Regulation of Innate Immune Responses by Bovine Herpesvirus 1 and Infected Cell Protein 0 (bICP0). J Viruses 2009; 1: 255-275.
[44] Lovato L, Inman M, Henderson G, Doster A, Jones C. Infection of cattle with a bovine herpesvirus 1 strain that contains a mutation in the latency-related gene leads to increased apoptosis in trigeminal ganglia during the transition from acute infection to latency. J Virology 2003; 7: 4848-4857.
[45] Jones C. Bovine Herpes Virus 1 (BHV-1) and Herpes simplex Virus Type 1 (HsV-1) promote survival of Latently Infected sensory neurons, in part by Inhibiting Apoptosis. J Cell Death 2013; 6: 1-16.
[46] Jones C. Regulation of innate immune responses by bovine herpesvirus 1 and infected cell protein 0. J Viruses 2009; 1: 255-275.
[47] De-la-Pena C, Lei Z, Watson BS, Sumner LW, Vivanco JM. Root-microbe communication through protein secretion. J Biol Chem 2008; 283: 25247-25255.
[48] Peng J, Zhang Q, Ma Y, Wang Y, Huang L, Zhang P, Chen J and Qin H. A rat-to-human search for proteomic alterations reveals transgelin as a biomarker relevant to colorectal carcinogenesis and liver metastasis. Electrophoresis 2009; 30: 2976-2987.
[49] Perkins DN, Pappin DJ, Creasy DM and Cottrell JS. Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis 1999; 20: 3551-3567.
[50] Koenig T, Menze BH, Kirchner M, Monigatti F, Parker KC, Patterson T, Steen JJ, Hamprecht FA and Steen H. Robust prediction of the MASCOT score for an improved quality assessment in mass spectrometric proteomics. J Proteome Res 2008; 7: 3708-3717.
[51] Pappin DJ, Hojrup P and Bleasby AJ. Rapid identification of proteins by peptide-mass fingerprinting. Curr Biol 1993; 3: 322-327.