P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Short Communication https://doi.org/10.47278/journal.ijvs/2025.005

Global Variants of Chicken Anemia Virus Apoptin as a Potential Anticancer Drug for Humans and Animals

Putu M. W. S. Putri [1], Febriyan M. Hartini [2], Harry P. Triwijayanto [2], Dianti Violentina [3], Hamong Suharsono [4], Ida B. K. Suardana [5], Made S. Kameswari [6], Nyoman B. Mahendra [7] and Gusti N. Mahardika [3,5]*

[1]Faculty of Medicine Udayana University, Jl. PB Sudirman, Denpasar – Bali, Indonesia
[2]Faculty of Veterinary Medicine Udayana University, Kampus Bukit Jimbaran, Badung – Bali, Indonesia
[3]Animal Biomedical and Molecular Biology Laboratory, Faculty of Veterinary Medicine Udayana University, Jl Sesetan-Markisa 6A, Denpasar – Bali, Indonesia
[4]Biochemistry Laboratory, Faculty of Veterinary Medicine Udayana University, Kampus Bukit Jimbaran, Badung – Bali, Indonesia
[5]Virology Laboratory, Faculty of Veterinary Medicine Udayana University, Kampus Bukit Jimbaran, Badung – Bali, Indonesia
[6]Faculty of Agriculture, Science, and Technology, Warmadewa University, Denpasar – Bali Indonesia
[7]Department of Obstetrics and Genecology, Faculty of Medicine Udayana University, Jl. PB Sudirman, Denpasar – Bali, Indonesia
***Corresponding author:** gnmahardika@unud.ac.id

Article History: 24-708 Received: 10-Nov-24 Revised: 31-Dec-24 Accepted: 01-Jan-25 Online First: 20-Jan-25

ABSTRACT

Apoptin from chicken anemia virus (CAV) is recognized for its anticancer potential. With over 800 available sequences, identifying the most effective variant is challenging. In this study, sequences were downloaded from GenBank, aligned via MEGA 11, and analyzed using Genesius Prime. Protein structure and database analysis were performed using Phyre 2. Results show an average genetic distance of 0.005, with 27 out of 121 residues were constant. The most frequent substitutions (S67N, L25S, V73A, C118R) were observed in varying frequencies across sequences. The artificial apoptin variant, featuring these substitutions, revealed an additional alpha-helix at the C-terminus. Phosphorylation and glycosylation patterns were mapped across sequences, with phosphorylation motifs identified at the peptide's termini. It is recommended that apoptin with intact phosphorylation and glycosylation motifs be prioritized for anticancer applications.

Key words: Apoptin; Apoptosis; Chicken anemia virus (CAV); Anticancer

INTRODUCTION

Apoptin, also known as viral protein 3 (VP3), is derived from the chicken anemia virus (CAV) and demonstrates promising anticancer properties due to its ability to selectively target cancerous cells (Los et al. 2009). This unique capability has been documented across a range of cancer types, including breast, liver, bone and colon cancers (Backendorf et al. 2008; Feng et al. 2020; Los et al. 2009).

The chicken anemia virus (CAV), a virus affecting poultry worldwide, is primarily known for inducing immunosuppression and anemia, particularly through its impact on lymphoid and hematopoietic cells (Fatoba and Adeleke 2019; Zhang et al. 2024). Historically classified under Circoviridae (https://ictv.global/report_9th/ssDNA/Circoviridae), CAV has been reclassified under Annelloviridae (Varsani et al. 2021; Yan et al. 2024). The CAV genome, comprising approximately 2200bp, includes three overlapping open reading frames (ORFs) coding for proteins associated with apoptosis and viral replication (Rosenberger and Cloud 1998; Lacorte et al. 2007). The apoptin protein, specifically coded by ORF2, selectively induces apoptosis in cancer cells without affecting normal cells (Koch et al. 1995; Yan et al. 2024).

Over 800 sequences of CAV apoptins are available in GenBank, providing numerous options for selecting the sequence with the strongest anticancer potential. The full-length genomes of hundreds of CAV strains have been analyzed, revealing that the global CAV has separated

Cite This Article as: Putri PMWS, Hartini FM, Triwijayanto HP, Violentina D, Suharsono H, Suardana IBK, Kameswari MS, Mahendra NB and Mahardika GN, 2025. Global variants of chicken anemia virus apoptin as a potential anticancer drug for humans and animals. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2025.005

into two major clades with 3-4 subclades (Shah et al. 2023). The variability coefficient of each viral protein was greater than 1, indicating considerable amino acid drift (Shah et al. 2023). Genetic variation in the CAV gene has been identified in the VP1, VP2, and VP3 genes (Abdel-Mawgod et al. 2024).

In this study, we analyzed all available apoptin sequences in GenBank, examining genetic distance, conserved and polymorphic amino acid residues, and evaluating both the 3-D and secondary structures of the consensus peptide alongside the sequence containing the most common substitutions. The findings aim to support future research in identifying the most effective apoptin sequence.

MATERIALS AND METHODS

All the VP3 sequence data of CAVs available in GenBank were downloaded on September 9, 2024. Each sequence was annotated with the accession number, country of origin, and identification year. The sequences were aligned via MEGA 11 software. All sequences with incorrect amino acid sequences were excluded. The sequences are listed on the basis of continent (Africa, Europe, Asia, Oceania, North America, and South America). Overall genetic distance was calculated via the Kimura 2-parameter model (Kimura 1980). Conserved and variable residues were manually tallied using the sequence data explorer output from MEGA 11, while consensus residues were identified through Geneious Prime software. N-linked glycosylation was performed following Rao and Bernd (2010) and O-linked glycosylation was performed following Pisano et al. (1993). Phosphorylation motives were scanned following Amanchy et al. (2011).

Analysis of the protein database and structural prediction for the consensus peptide, in comparison with the most common substitution pattern in the putative sequence, were performed using the online tool Phyre 2 (Bennett-Lovsey et al. 2008; Kelley and Sternberg 2009).

RESULTS

The distribution and year of isolation or identification of CAV sequence data available in GenBank are presented in Table 1. Analysis revealed 828 global CAV sequences, which are predominantly from Asia (713). Data from Africa dated before 2010, America after 2021 and Oceania after 2011 were not available.

Table 1: Global distribution and year of isolation or identification of CAV sequence data

	Year of Isolation or identification				
Location	Up to 2000	2001-2010	2011-2020	From 2021	Total
Africa	0	0	39	4	43
America (north and south)	4	8	9	0	21
Asia	10	51	421	231	713
Europe	12	2	16	7	37
Oceania	1	11	0	0	12

The overall mean distance was 0.005. The conserved residues were M1, S13, E32, G36, L44, S45, G48, A50, T56, L57, R58, S59, A60, T61, D63, G69, L76, Q80, P83, P84, S85, R88, P92, S93, L100, T107 and P109. The consensus peptide showed no glycosylation patterns of NXS or NXT; however, these patterns appeared in a few isolates. Many O-linked glycosylation patterns of XPXX, in which at least one X is T; TXXX, in which at least one X is T; XXTX, in which at least one R or K; and SXXX, in which at least one X is S, following reference Pisano et al. (1993), are spread across all sequences. SXXXXTP is phosphorylated at the amino and carboxy termini of the consensus peptide, whereas TPXXXXXR is phosphorylated at the amino terminus.

Table 2 presents the number of strains harboring substituted residues of CAV differs from that harboring consensus apoptin which occurred in five sequence data or more. The four most prevalent substitutions, ranked by frequency, were S67N, L25S, V73A and C118R, occurring in 91, 51, 34, and 32 sequences, respectively.

Table 2: The number of strains harboring substituted residues of CAV differs from that harboring consensus apoptin

Position	Residue in the most strain	Substitution*	number of strains harboring the substituted residue
2	N	S	5
3	A	G	9
4**	L	P/H	11/5
6	E	D	7
8	T	S	7
12	P	Q	5
19	P	A	7
23	R	Q	10
25	L	S	51
31	R	K	12
52	A	V	7
67	S	N	91
70	F	S	5
73	V	A	34
79	D	N	5
98	S	N	17
103	S	N	17
108	T	A	12
116	R	K	17
118	C	R	32

*Only substitutions that occurred in five sequences or higher were counted. ** substitution L4P occurred in 11 sequences, L4H in 5.

Cartoon peptide modelling of apoptin in the consensus sequence and artificial sequence with the four most frequent substitutions is presented in Fig. 1. A search within the protein database yielded no matches with confidence levels exceeding 5%. Fig. 2 displays the 3-D protein secondary structure predictions for the consensus peptide alongside the putative sequence containing the four most common substitutions. Both figures indicate that the modified apoptin includes an additional alpha-helix at the carboxy end.

DISCUSSION

The selective killing of cancer cells is a prominent feature of apoptin as a breakthrough modality in cancer treatment in humans and animals. Although there have been no reports of the application of apoptin in animals, we believe that this method should also be an effective modality for cancer therapy in pet animals. Cancer causes pet animal suffering (Misdorp 1996; Merlo et al. 2008; Di Cerbo et al. 2014; Baioni et al. 2017). The risk of suffering neoplasia, especially in old dogs and cats are high (https://www.avma.org/).

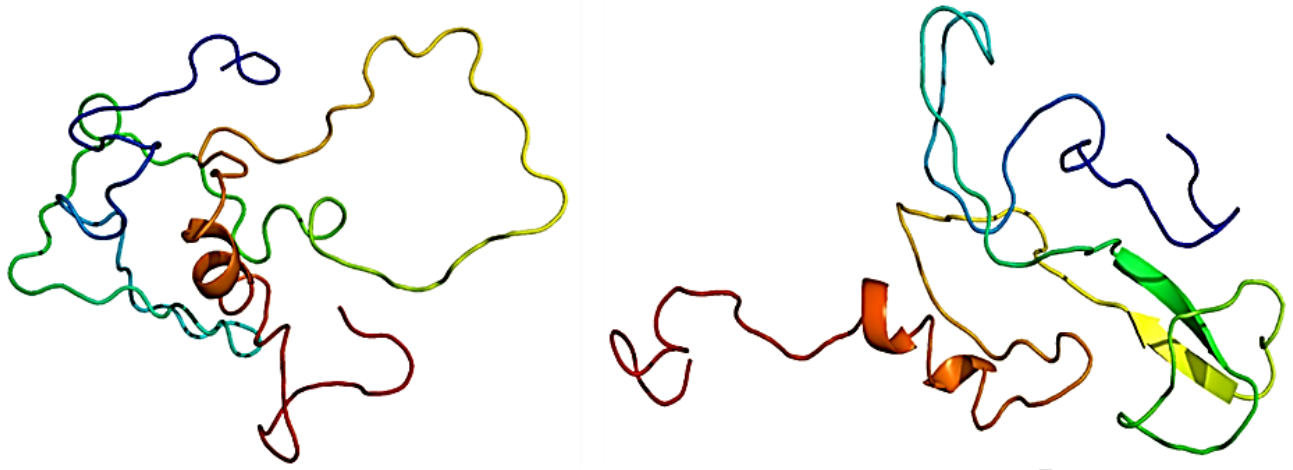

Fig. 1: Cartoon peptide modeling of apoptin in the consensus sequence (left) and artificial sequence with the four most frequent substitutions (right). The images are colored in an inverted rainbow from the N- to the C-terminus. Protein modeling was performed with the online resource PHYRE2 (http://www.sbg.bio.ic.ac.uk) (Bennett-Lovsey et al. 2008; Kelley and Sternberg 2009). Protein models were visualized with RasWin 2.7.5.2 (www.rasmol.org).

Fig. 2: Secondary structure and disorder prediction of apoptin in the consensus sequence (above) and artificial sequence with the four most frequent substitutions (below). Protein secondary structure and disorder prediction was performed with the online resource PHYRE2 (http://www.sbg.bio.ic.ac.uk) (Bennett-Lovsey et al. 2008; Kelley and Sternberg 2009).

Some of the key advantages of apoptin as an anticancer agent are, first, the selective targeting of cancer cells: Apoptin activates apoptotic pathways specifically in cancer cells, largely through increased levels of protein kinases in tumors (Caretta and Mucignat-Caretta 2011). Second, apoptin activity is independent of p53 (Jeurissen et al. 1992; Zhuang et al. 1995; Danen-Van Oorschot et al. 1997). In addition, apoptin shows minimal toxicity to healthy cells (Danen-Van Oorschot et al. 1997; Malla et al. 2020). Moreover, apoptin has been proven to have a wide range of effectiveness in diverse cancer types (Tavassoli et al. 2005; Malla et al. 2020). Finally, apoptin can be combined with other therapies to overcome resistance to anticancer therapy (Bayat Mokhtari et al. 2017).

In selecting the most potent anticancer agent of apoptin, more than 800 sequences are available in GenBank. The CAVs originated from all continents but were mostly from Asia and were identified from 2011-2020. Interestingly, the overall genetic distance was very low, but only 27 out of 121 (22.3%) conserved residues were present. Some of these effects might be related to the biological function of apoptin. The most frequent substitutions were S67N, L25S, V73A, and C118R, occurring in 11.0, 6.2, 4.1, and 3.9% of the 828 data, respectively. The global distribution of sequences with S67N and L25S mutations has occurred on all continents since 1999. V73A has been detected in all continents except Europe since 2003. C188R emerged on all continents except Oceania in 1997. These substitutions seem to have occurred not only recently. In addition, the well-identified phosphorylation site of T108 (Rohn et al. 2002; Lee et al. 2007) was not conserved in our dataset. There were 12 strains with A (Table 2) and two with S. If T108 is indeed critical for phosphorylation, strains with aberrant residues at that site might express less anticancer activity.

Apoptin seems to be novel, as a protein database search resulted in no hits with confidence > 5%. Protein 3-D and peptide secondary structure prediction revealed that the artificial apoptin has an additional alpha-helix structure (Fig. 1 and 2). This might affect the anticancer activity of the respective peptide.

We described here the present N-link and O-link glycosylation patterns. The glycosylation motives of apoptin have yet to be investigated. The consensus deduced amino acid sequence in our dataset indeed harbors no N-Link glycosylation motif for NXS/NXT (Rao and Bernd 2010). However, the NXS pattern occurred four times, whereas NXT occurred once across our dataset. The O-linked glycosylation motif was present in the consensus sequence and in all strains. The patterns XPXX in which at least one X is T, TXXX in which at least one X is T, XXTX in which at least one X is R or K, and SXXX in which at least one X is S (Pisano et al. 1993) occurred 7, 3, 2, and 2 times in the consensus, respectively. If glycosylation does occur in natural apoptin, some amino acid substitutions across the strains might affect the biological function of apoptin, including its anticancer capacity.

Glycosylation is an important protein translational modification (PTM) process that involves aspartate, S/T or Y residues that target N- and O-glycosylation, respectively (He et al. 2024). It plays a role in protein folding, protein stability, and protein–protein interactions (Mustafa and Komatsu 2014). Changes in glycosylation have been shown to alter the function of respective proteins (Reily et al. 2019; He et al. 2024). Although it has yet to be discovered, glycosylated apoptin might be involved in many processes, especially O-linked glycosylation.

Other PTMs of phosphorylation should be considered in the selection of apoptin. Out of hundreds of phosphorylation motifs (Amanchy et al. 2011), we demonstrated at least two patterns, i.e., SXXXXTP was located at both ends of the consensus peptide, whereas TPXXXXXR occurred once at the proximal end. The four most common substitutions do not lead to the loss of these motives.

Many aspects of apoptin need further investigation. Indirect evidence should be provided by the history of the clinical course of the respective CAV strains in chickens. Metadata of each sequence are not available. As of now, selecting the complete apoptin with intact phosphorylation and glycosylation motifs is essential before identifying the most effective sequence that exhibits strong anticancer effects.

Because its coding and amino acid sequences are well known, many platforms and methods of administration are available. The first option is synthetic peptides. With only 121 amino acids, apoptin can be synthesized. This could be the whole apoptin or minimal region of the apoptin domain required for certain effects on cancer regression (Jangamreddy et al. 2014; Noei et al. 2019; Zhang et al. 2017). The next platform is plasmid DNA (Han et al. 2008) or vectored apoptin (Kochneva et al. 2013; Backendorf and Noteborn 2014; Song et al. 2021), which can be produced as a fusion protein with other anticancer peptides.

We conclude that the degree of CAV apoptosis varies. Among the 121 amino acid sequences of CAV strains worldwide, only 22.3% (27 residues) were conserved. The four most common substitutions were S67N, L25S, V73A, and C118R. The four most common substitutions do not lead to damage to the identified phosphorylation motifs.

Data availability: The complete list of GenBank accession numbers, countries of origin, isolation and identification years as well as the fasta files of all the data as well as the continent-based datasets are available upon request.

Conflict of Interest: The authors claim that they have no conflicts of interest.

Acknowledgment: The work was supported by the Animal Biomedical Molecular Biology Laboratory and the Indonesian Biodiversity Research Center of Udayana University, Denpasar, Bali, Indonesia. Some issues and literature searches were generated via the artificial intelligence machine ChatGPT. We have carefully validated the AI results. English editing was conducted by Curie's AI.

Author's Contribution: The idea was developed by PMWSP, FMH, HPT and GNM. PMWSP, FMH, HPT, and DV collected and compiled the data. HS, IBKS, MSK, and NBM contributed to the literature search and data analysis. IBKS and GNM drafted the manuscript. All the authors have read the final document and agreed to be published.

REFERENCES

Abdel-Mawgod S, Zanaty A, Elhusseiny M, Said D, Samir A, Elsayed MM, Mahana O, Said M, Hussein AM, Hassan HM, Selim A, Shahien MA and Selim K, 2024. Genetic heterogeneity of chicken anemia virus isolated in selected Egyptian provinces as a preliminary investigation. Frontiers in Veterinary Science 11: 1362219. https://doi.org/10.3389/fvets.2024.1362219

Amanchy R, Kandasamy K, Mathivanan S, Periaswamy B, Reddy R, Yoon WH, Joore J, Beer MA, Cope L and Pandey A, 2011. Identification of novel phosphorylation motifs through an integrative computational and experimental analysis of the human phosphoproteome. Journal of Proteomics and

Bioinformatics 4: 22-35. https://doi.org/10.4172/jpb.1000163

Backendorf C and Noteborn MH, 2014. Apoptin towards safe and efficient anticancer therapies. Advances in Experimental Medicine and Biology 818: 39-59. https://doi.org/10.1007/978-1-4471-6458-6_3

Backendorf C, Visser AE, de Boer AG, Zimmerman R, Visser M, Voskamp P, Zhang YH and Noteborn M, 2008. Apoptin: therapeutic potential of an early sensor of carcinogenic transformation. The Annual Review of Pharmacology and Toxicology 48: 143-169. https://doi.org/10.1146/annurev.pharmtox.48.121806.154910

Baioni E, Scanziani E, Vincenti MC, Leschiera M, Bozzetta E, Pezzolato M, Desiato R, Bertolini S, Maurella C and Ru G, 2017. Estimating canine cancer incidence: findings from a population-based tumour registry in northwestern Italy. BMC Veterinary Research 13: 203. https://doi.org/10.1186/s12917-017-1126-0

Bayat MR, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B and Yeger H, 2017. Combination therapy in combating cancer. Oncotarget 8: 38022-38043. https://doi.org/10.18632/oncotarget.16723

Bennett-Lovsey RM, Herbert AD, Sternberg MJ and Kelley LA, 2008. Exploring the extremes of sequence/structure space with ensemble fold recognition in the program Phyre. Proteins 70: 611-625. https://doi.org/10.1002/prot.21688

Caretta A and Mucignat-Caretta C, 2011. Protein kinase a in cancer. Cancers (Basel) 3: 913-926. https://doi.org/10.3390/cancers3010913

Danen-Van Oorschot AA, Fischer DF, Grimbergen JM, Klein B, Zhuang S, Falkenburg JH, Backendorf C, Quax PH, Van der Eb AJ and Noteborn MH, 1997. Apoptin induces apoptosis in human transformed and malignant cells but not in normal cells. The Proceedings of the National Academy of Sciences U S A 94: 5843-5847. https://doi.org/10.1073/pnas.94.11.5843

Di Cerbo A, Palmieri B, De Vico G and Iannitti T, 2014. Onco-epidemiology of domestic animals and targeted therapeutic attempts: perspectives on human oncology. Journal of Cancer Research and Clinical Oncology 140: 1807-1814. https://doi.org/10.1007/s00432-014-1664-9

Fatoba AJ and Adeleke MA, 2019. Chicken anemia virus: A deadly pathogen of poultry. Acta Virologica 63: 19-25. https://doi.org/10.4149/av_2019_110

Feng C, Liang Y and Teodoro JG, 2020. The Role of Apoptin in Chicken Anemia Virus Replication. Pathogens 9. https://doi.org/10.3390/pathogens9040294

Han SX, Ma JL, Lu Y, Huang C and Duan KM, 2008. [SP-TAT-Apoptin induces G1 arrest in HepG2 cells]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 24: 864-866

He M, Zhou X and Wang X, 2024. Glycosylation: mechanisms, biological functions and clinical implications. Signal Transduction and Targeted Therapy 9: 194. https://doi.org/10.1038/s41392-024-01886-1

Jangamreddy JR, Panigrahi S, Lotfi K, Yadav M, Maddika S, Tripathi AK, Sanyal S and Los MJ, 2014. Mapping of apoptin-interaction with BCR-ABL1, and development of apoptin-based targeted therapy. Oncotarget 5: 7198-7211. https://doi.org/10.18632/oncotarget.2278

Jeurissen SH, Wagenaar F, Pol JM, van der Eb AJ and Noteborn MH, 1992. Chicken anemia virus causes apoptosis of thymocytes after in vivo infection and of cell lines after in vitro infection. Journal of Virology 66: 7383-7388. https://doi.org/10.1128/JVI.66.12.7383-7388.1992

Kelley LA and Sternberg MJ, 2009. Protein structure prediction on the Web: a case study using the Phyre server. Nature Protocols 4: 363-371. https://doi.org/10.1038/nprot.2009.2

Kimura M, 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111-120

Koch G, van Roozelaar DJ, Verschueren CA, van der Eb AJ and Noteborn MH, 1995. Immunogenic and protective properties of chicken anaemia virus proteins expressed by baculovirus. Vaccine 13: 763-770. https://doi.org/10.1016/0264-410x(94)00034-k

Kochneva GV, Babkina IN, Lupan TA, Grazhdantseva AA, Iudin PV, Sivolobova GF, Shvalov AN, Popov EG, Babkin IV, Netesov SV and Chumakov PM, 2013. [Apoptin enhances the oncolytic activity of vaccinia virus]. Molecular Biology (Moscow) 47: 842-852

Lacorte C, Lohuis H, Goldbach R and Prins M, 2007. Assessing the expression of chicken anemia virus proteins in plants. Virus Research 129: 80-86. https://doi.org/10.1016/j.virusres.2007.06.020

Lee YH, Cheng CM, Chang YF, Wang TY and Yuo CY, 2007. Apoptin T108 phosphorylation is not required for its tumor-specific nuclear localization but partially affects its apoptotic activity. Biochemical and Biophysical Research Communications 354: 391-395. https://doi.org/10.1016/j.bbrc.2006.12.201

Los M, Panigrahi S, Rashedi I, Mandal S, Stetefeld J, Essmann F and Schulze-Osthoff K, 2009. Apoptin, a tumor-selective killer. Biochimica et Biophysica Acta 1793: 1335-1342. 10.1016/j.bbamcr.2009.04.002

Malla WA, Arora R, Khan RIN, Mahajan S and Tiwari AK, 2020. Apoptin as a Tumor-Specific Therapeutic Agent: Current Perspective on Mechanism of Action and Delivery Systems. Frontiers in Cell and Developmental Biology 8: 524. https://doi.org/10.3389/fcell.2020.00524

Merlo DF, Rossi L, Pellegrino C, Ceppi M, Cardellino U, Capurro C, Ratto A, Sambucco PL, Sestito V, Tanara G and Bocchini V, 2008. Cancer incidence in pet dogs: findings of the Animal Tumor Registry of Genoa, Italy. Journal of Veterinary Internal Medicine 22: 976-984. https://doi.org/10.1111/j.1939-1676.2008.0133.x

Misdorp W, 1996. Veterinary cancer epidemiology. Veterinary Quarterly 18: 32-36. https://doi.org/10.1080/01652176.1996.9694610

Mustafa G and Komatsu S, 2014. Quantitative proteomics reveals the effect of protein glycosylation in soybean root under flooding stress. Frontiers in Plant Science 5: 627. https://doi.org/10.3389/fpls.2014.00627

Noei A, Nili-Ahmadabadi A and Soleimani M, 2019. The Enhanced Cytotoxic Effects of the p28-Apoptin Chimeric Protein As A Novel Anti-Cancer Agent on Breast Cancer Cell Lines. Drug Research (Stuttgart) 69: 144-150. https://doi.org/10.1055/a-0654-4952

Pisano A, Redmond JW, Williams KL and Gooley AA, 1993. Glycosylation sites identified by solid-phase Edman degradation: O-linked glycosylation motifs on human glycophorin A. Glycobiology 3: 429-435. 10.1093/glycob/3.5.429

Rao RS and Bernd W, 2010. Do N-glycoproteins have preference for specific sequons? Bioinformation 5: 208-212. https://doi.org/10.6026/97320630005208

Reily C, Stewart TJ, Renfrow MB and Novak J, 2019. Glycosylation in health and disease. Nature Reviews Nephrology 15: 346-366. https://doi.org/10.1038/s41581-019-0129-4

Rohn JL, Zhang YH, Aalbers RI, Otto N, Den Hertog J, Henriquez NV, Van De Velde CJ, Kuppen PJ, Mumberg D, Donner P and Noteborn MH, 2002. A tumor-specific kinase activity regulates the viral death protein Apoptin. The Journal of Biological Chemistry 277: 50820-50827. https://doi.org/10.1074/jbc.M208557200

Rosenberger JK and Cloud SS, 1998. Chicken anemia virus. Poultry Science 77: 1190-1192. https://doi.org/10.1093/ps/77.8.1190

Shah PT, Bahoussi AN, Cui X, Shabir S, Wu C and Xing L, 2023. Genetic diversity, distribution, and evolution of chicken

anemia virus: A comparative genomic and phylogenetic analysis. Frontiers in Microbiology 14: 1145225. https://doi.org/10.3389/fmicb.2023.1145225

Song G, Fang J, Shang C, Li Y, Zhu Y, Xiu Z, Sun L, Jin N and Li X, 2021. Ad-apoptin inhibits glycolysis, migration and invasion in lung cancer cells targeting AMPK/mTOR signaling pathway. Experimental Cell Research 409: 112926. 10.1016/j.yexcr.2021.112926

Tavassoli M, Guelen L, Luxon BA and Gaken J, 2005. Apoptin: specific killer of tumor cells? Apoptosis 10: 717-724. https://doi.org/10.1007/s10495-005-0930-3

Varsani A, Opriessnig T, Celer V, Maggi F, Okamoto H, Blomstrom AL, Cadar D, Harrach B, Biagini P and Kraberger S, 2021. Taxonomic update for mammalian anelloviruses (family Anelloviridae). Archives of Virology 166: 2943-2953. https://doi.org/10.1007/s00705-021-05192-x

Yan T, Wang Z, Li R, Zhang D, Song Y and Cheng Z, 2024. Gyrovirus: current status and challenge. Frontiers in Microbiology 15: 1449814. https://doi.org/10.3389/fmicb.2024.1449814

Zhang J, Ma L, Li T, Xie Q, Wan Z, Qin A, Ye J, Shao H and Wang S, 2024. Isolation and genomic characterization of chicken infectious anemia virus in Jiangsu province of China during 2020-2022. Frontier Veterinary Science 11: 1378120. https://doi.org/10.3389/fvets.2024.1378120

Zhang L, Zhao H, Cui Z, Lv Y, Zhang W, Ma X, Zhang J, Sun B, Zhou D and Yuan L, 2017. A peptide derived from apoptin inhibits glioma growth. Oncotarget 8: 31119-31132. https://doi.org/10.18632/oncotarget.16094

Zhuang SM, Shvarts A, van Ormondt H, Jochemsen AG, van der Eb AJ and Noteborn MH, 1995. Apoptin, a protein derived from chicken anemia virus, induces p53-independent apoptosis in human osteosarcoma cells. Cancer Research 55: 486-489