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Intratumoral virotherapy with Indonesian Newcastle disease virus reduces mTOR expression and induces apoptosis in rat breast cancer models

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ABSTRACT

Autophagy and apoptosis are two key cellular pathways that are commonly promoted as therapeutic strategies for inhibiting cancer growth. Newcastle disease virus (NDV) has shown strong potency as cancer therapeutic agent and has been reported to induce both autophagy and apoptosis in malignant cells. NDV isolate from Indonesia has demonstrated oncolytic activity; however, the underlying mechanisms are not yet fully understood. Therefore, this study aimed to investigate the oncolytic mechanism of the Indonesian NDV isolate Tabanan-1/ARP/2017 by examining the expression of mTOR and caspase-3. Paraffin-embedded tissue blocks were obtained from a previous study. Six rats with breast cancer were divided into two groups: placebo group receiving 0.5 mL PBS, and therapy group receiving 7 $\log_2$ HAU of NDV Tabanan-1/ARP/2017 per 0.5 mL administered intratumorally. Treatments were performed once daily for four consecutive days. After 15 days of initial treatment, the tumor tissues were collected, processed into paraffin blocks, and immunohistochemically stained with anti-mTOR and anti-caspase-3. The expression of both proteins was analyzed based on optical density using Fiji (ImageJ). mTOR expression in the therapy group was significantly lower than in the placebo group, whereas caspase-3 expression was significantly higher in the therapy group. These findings indicate that NDV Tabanan-1/ARP/2017 therapy is associated with decreased mTOR expression and apoptosis induction, as indicated by caspase-3 activation.

Introduction

Recently, there has been increased interest in virotherapy as cancer treatment that use oncolytic viruses to selectively kill cancer cells (Lin *et al*., 2023). This approach represents as a potential alternative to conventional therapy, such as chemotherapy that causes damage to non-targeted cells such as healthy cells (Juthani *et al*., 2024). The high selectivity of these viruses toward cancer cells is attributed to the presence of specific receptors on the cancer cell surface and a tumor microenvironment that supports viral replication (Al-Shammari and Salman, 2024). Newcastle disease virus (NDV) is considered as strong potential cancer virotherapy agent because it offers several advantages, including being non-pathogenic to humans and posing minimal health risks to patients. Moreover, it exhibits strong selectivity for cancer cells and does not exert cytotoxic effects on normal cells (Ganar *et al*., 2014).

The NDV Tabanan-1/ARP/2017 from Indonesia has been reported to possess oncolytic potential. Previous studies have demonstrated its ability to suppress fibrosarcoma growth in mice and rat models (Sewoyo *et al*., 2021; Rakhmawati *et al*., 2022), as well as to inhibit breast cancer progression (Sewoyo *et al*., 2024; Sewoyo *et al*., 2026). Further research has shown that this virus is able to reduce the expression of several biomarkers, including Ki67 and VEGF in rat breast cancer models (Sewoyo *et al*., 2026). The reduction of these proteins' expression might be mediated by apoptosis and autophagy induced by the virus.

Many have reported that NDV induces autophagy in cancer cells (Meng *et al*., 2012; Bu *et al*., 2015; Ye *et al*., 2018; Kan *et al*., 2021). Autophagy is a macromolecular degradation process that primarily targets damaged proteins and organelles (Pyo *et al*., 2012). One of the signaling pathways involved in autophagy regulation is the mTORC1-ULK1-AMPK triangle. AMPK phosphorylates ULK1 at Ser555, thereby activating autophagy, whereas mTORC1 phosphorylates ULK1 at Ser757, which leads to the inhibition of autophagy (Gui *et al*., 2017; Wang *et al*., 2022). The activated AMPK also contributed to decreased mTORC1 activity (Ren *et al*., 2019). Suppression of mTORC1 releases this inhibition and allows autophagy to proceed. In addition to autophagy, several studies have also shown that the induction of apoptosis by NDV contributes to the inhibition of malignant tumor growth (Ghrici *et al*., 2013; Al-Shammari *et al*., 2014; Wang *et al*., 2023; Al-Shammari and Salman, 2024). It is known that one of cancer hallmarks is resistance to apoptosis (Hanahan, 2022), so inducing apoptosis in cancer cells would be beneficial to reduce its growth.

Here we further investigated the oncolytic mechanism of NDV Tabanan-1/ARP/2017 in rat breast cancer models by assessing mTOR and caspase-3 expression.

Materials and methods

Ethics Permission and Study Designs

This study received approval from the ethics committee of our faculty under permit number B/113/UN14.2.9/PT.01.04/2025. Paraffin-embedded tissue blocks were obtained from a previous study (Sewoyo *et al*., 2026). Six virgin female Sprague Dawley rats with breast cancer (ductal carcinoma *in situ*) experimentally induced with 7,12-dimethylbenz(α)anthracene were divided into two groups: placebo and therapy. Rats in the placebo group received 0.5 mL of sterile PBS, while those in the therapy group were administered NDV Tabanan-1/ARP/2017 at a dose of 7 $\log_2$ hemagglutination units (HAU)/0.5 mL. Both treatments were delivered intratumorally once daily for four consecutive days. The observation period is 15 days from the first day of treatment.

Immunohistochemistry

The paraffin-embedded tissue blocks were sectioned at a thickness of 5 µm, then mounted on glass slides and subjected to deparaffiniza-

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tion. Deparaffinization was performed using xylol I and II, each for 5 min, followed by air drying for 10 min. The sections were then rehydrated through a graded alcohol series (100% and 96%, 5 min each) and rinsed with distilled water.

Antigen retrieval was conducted using a combined heat and enzymatic method by heating the slides in microwave (95°C) for 30 min in citrate buffer. H2O2 (3%) in methanol was used to block endogenous peroxidase. The primary antibodies used were anti-mTOR polyclonal antibody (Catalog No. bs-3494R, Bioss Inc., USA), diluted at 1:50 and anti-caspase-3 monoclonal antibody (Catalog No. bsm-552297R, Bioss Inc., USA), diluted at 1:100. The sections were incubated with the primary antibodies overnight at room temperature, then washed twice with PBS for 4 min each. Subsequently, anti-mouse and -rabbit polymer conjugated with horseradish peroxidase (Catalog No. 414151F, Nichirei Biosciences Inc., Japan) was applied then incubated for 1 h at room temperature. The slides were then washed with PBS. Diaminobenzidine (DAB) was used as the chromogen and incubated for 5 min to visualize antigen-antibody reactions.

Counterstaining was performed using Mayer's hematoxylin. The sections were dehydrated through 96% and 100% ethanol (5 min each), cleared in two changes of xylol (5 min each), and finally mounted with Entellan® and covered with a coverslip.

mTOR and Caspase-3 Assessment

Observations were carried out using an Olympus CX53 microscope equipped with digital camera (OptiLab®). Images were captured and analyzed using Fiji (ImageJ) software. The initial step of image processing involved color deconvolution to separate the DAB and hematoxylin staining. Quantitative analysis was then performed on the DAB-stained images that converted into 8-bit. Optical density (OD) was calculated using the formula in which OD equals the logarithm of the ratio between the maximum intensity and the image intensity, where the maximum intensity for 8-bit images was set to 255 (Akram *et al*., 2023). Observations were made in five different fields of view at 400× magnification, and the OD values were then calculated each field and averaged to represent the expression for each sample.

Statistical Analysis

The OD values for mTOR and caspase-3 were analyzed using an independent t-test with Statistical Package for the Social Sciences (IBM) for Windows. A p-value<0.05 was considered statistically significant.

Results

The mTOR expression was evaluated using immunohistochemistry in both the placebo and therapy groups. Positive cells against anti-mTOR were observed in the cytoplasm, appeared brown in color. Placebo group showed a stronger brown staining intensity compared to the therapy group (Figure 1a,b). The OD value in the therapy group was significantly lower than that in the placebo group, and statistical analysis using t-test showed a significant difference (p<0.05, Figure 1c, Table 1).

Positive cells against anti-caspase-3 were observed in both nucleus and cytoplasm, appeared brown in color. The therapy group showed a stronger brown staining intensity, while placebo group almost none were

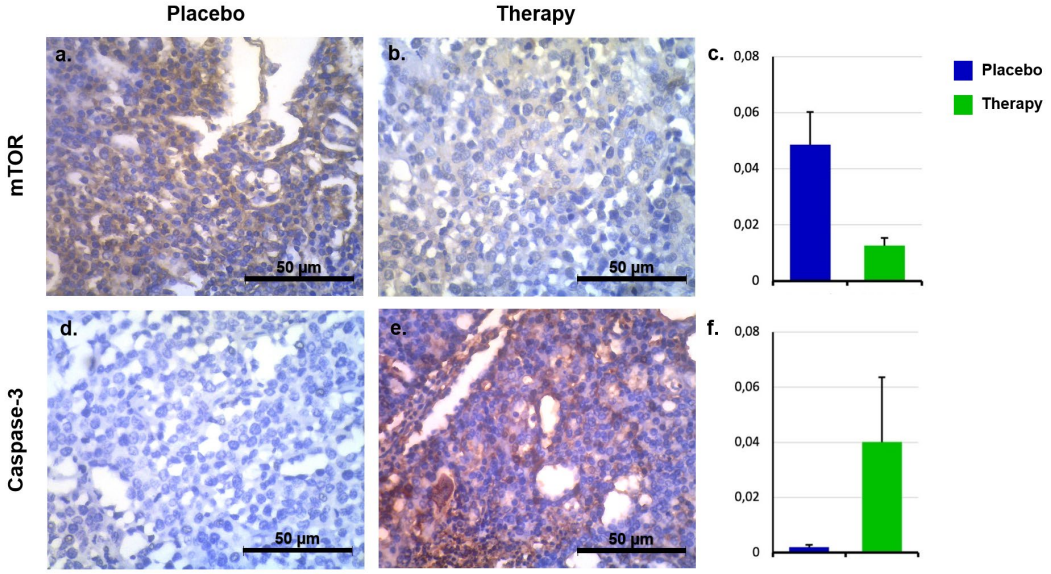

Figure 1. Immunohistochemical staining with anti-mTOR (a, b) and anti-caspase-3 (d, e). (a) Placebo group showing intense and more extensive mTOR staining compared to (b) the therapy group. (c) OD values of mTOR expression between the placebo and therapy groups. (d) Caspase-3 was not expressed in tumor cells of the placebo group, whereas (e) the therapy group showed several positive cells. (f) OD values of caspase-3 expression between the placebo and therapy groups.

Table 1. The optical density (OD) of mTOR and caspase-3 in each group of treatments.

Treatment	Rat no.	mTOR	Significance	Caspase-3	Significance
Placebo	1	0.035±0.020	0.028*	0.002±0.001	0.048*
	2	0.050±0.020		0.001±0.001	
	3	0.058±0.029		0.002±0.001	
Therapy	1	0.010±0.005		0.056±0.002	
	2	0.015±0.004		0.051±0.001	
	3	0.011±0.002		0.013±0.001	

Note: Placebo group was injected intratumorally with 0.5 mL PBS, whereas therapy group injected with NDV Tabanan-1/ARP/2017 7 log2 HAU/0.5 mL. *Significance level at p<0.05. Analyzed with independent t-test.

positive against anti-caspase-3 (Figure 1d,e). The OD value in the therapy group was significantly higher than that in the placebo group, and statistical analysis using t-test showed a significant difference ($p<0.05$, Figure 1f, Table 1).

Discussion

The mTOR is a central regulator of cell growth and metabolism, playing an important role in autophagy and maintaining cellular homeostasis (Forbes *et al*., 2011). Overactivation of mTOR occurs in approximately 70% of breast cancer cases and has been shown to contribute to tumorigenesis in both animal models and clinical cancer patients (Ciuffreda *et al*., 2010; Lopez-Knowles *et al*., 2010). Because of its role in cancer progression, the mTOR pathway represents a key target for cancer therapy. Inhibition of mTOR has been reported to increase apoptosis in cancer cells and trigger autophagy, thereby helping to suppress cancer progression (He *et al*., 2016; Pyo *et al*., 2012; Yun and Lee, 2018). However, it is noteworthy that autophagy plays dual role in cancer, both tumor suppression and promotor. Promoting autophagy can be beneficial in the early stage of cancers and in apoptosis-defective cells, as autophagy can induce cell death. Furthermore, autophagy can limit invasion and tumor cells dissemination from primary site (Bhutia *et al*., 2013).

In this study, rats treated with NDV Tabanan-1/ARP/2017 showed significantly lower mTOR expression compared to group that injected with PBS. Ren *et al*. (2019) reported that the F and HN proteins of NDV can suppress mTORC1 expression in A549 lung adenocarcinoma cells. Both proteins induce cell membrane fusion, which activates AMPK. The activated AMPK phosphorylates and inactivates Raptor, a component of the mTORC1 complex, leading to decreased mTORC1 activity (Ren *et al*., 2019). As previously mentioned, increased AMPK activity also phosphorylates ULK1 at Ser555, triggering autophagy activation. On the other hand, suppression of mTORC1 leads to dephosphorylation of ULK1 at Ser757, thereby releasing the inhibition of autophagy (Gui *et al*., 2017).

Another study by Jiang *et al*. (2014) demonstrated that NDV/FMW infection in paclitaxel-resistant A549 lung adenocarcinoma cells decreased PI3K/Akt/mTOR/p70S6K pathway activity, inducing autophagy and enhancing the oncolytic effect. Similarly, Kim *et al*. (2024) reported that infection with recombinant NDV carrying the PTEN gene in pancreatic cancer models with KRAS mutations significantly reduced PI3K/AKT/mTOR pathway activity. This reduction not only suppressed tumor proliferation but also increased apoptosis and inhibited tumor growth in vivo. Based on these findings, the observed suppression of mTOR expression suggests a potential involvement of autophagy-related pathways. However, no direct evidence of autophagy was assessed in this study, and therefore, autophagy induction cannot be concluded. Further studies examining autophagy-specific markers such as ULK1, Beclin-1, and LC3 are required to confirm this mechanism. Additional study is needed to explain the specific signaling pathways involved in mTOR inhibition by the virus. A reduction in mTOR expression following NDV therapy has not been previously documented in breast cancer, making the findings of this study novel.

This study showed increased caspase-3 expression following treatment with NDV Tabanan-1/ARP/2017 compared to group that injected with PBS. Caspase-3 is a key pro-apoptotic protein that functions as a general marker of apoptosis (Noor *et al*., 2022). It acts as an apoptosis executioner, activated by caspase-8 in the extrinsic pathway or by caspase-9 in the intrinsic pathway (Boland *et al*., 2013). Many NDV isolates has been reported to induce apoptosis through both pathways (Elankumaran *et al*., 2006). In the intrinsic pathway, NDV infection promotes the release of cytochrome c, which subsequently activates caspase-9 and then caspase-3/7 to execute apoptosis. In the extrinsic pathway, NDV induces apoptosis via the production of TRAIL and TNF-α, which activate caspase-8, leading to the downstream activation of caspase-3/7 (Pathak *et al*., 2023).

Ghrici *et al*. (2013) reported that NDV AF2240 rapidly activated the apoptotic pathway through caspase-8 activation within two hours of infection, followed by increased caspase-3 in MCF-7 breast cancer cells, even before viral protein synthesis occurred. This early activation indicates that apoptosis can occur independently of viral replication. Similarly, Wang *et al*. (2023) demonstrated that the NDV LaSota strain increased the expression of cleaved poly-[ADP-ribose]-polymerase (PARP), caspase-3, and caspase-8 in canine mammary carcinoma cells both in vitro and in vivo through TNF-α/NF-κB pathway, leading to apoptosis induction. An increase in caspase-3 expression was also reported by Al-Shammari *et al*. (2014), that local Iraqi NDV strain administered intratumorally to mouse mammary adenocarcinoma models resulted in significant caspase-3 expression and extensive necrosis-apoptosis areas up to 14 days post-infection. Furthermore, Al-Shammari and Salman (2024) found that the NDV strain AMHA1 effectively induced apoptosis in metastatic breast cancer cells through caspase-3 activation and proliferation suppression, as indicated by decreased Ki67 expression, without causing damage to normal cells. Together, these findings confirm that apoptosis induction is one of the key mechanisms of NDV in suppressing malignant tumor growth, as also demonstrated by NDV Tabanan-1/ARP/2017 in this study. Further research is needed to determine whether NDV Tabanan-1/ARP/2017 induces apoptosis through the intrinsic pathway, the extrinsic pathway, or both.

Conclusion

Intratumoral therapy of NDV Tabanan-1/ARP/2017 in rat breast cancer models is associated with decreased mTOR expression and induction of apoptosis, as indicated by caspase-3 activation.

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Conflict of interest

The authors have no conflict of interest to declare.

References

Akram, S., Al-Shammari, A.M., Sahib, H.B., Jabir, M.S., 2023. Papaverine enhances the oncolytic effects of Newcastle disease virus on breast cancer in vitro and in vivo. Int. J. Microbiol. 2023, 17.

Al-Shammari, A.M., Humadi, T.J., Al-Taee, E.H., Yaseen, N.Y., 2014. Caspase-3 apoptotic induction by Iraqi Newcastle disease virus on mammary adenocarcinoma transplanted in mice. Iraqi J. Cancer Med. Genet. 7, 56-64.

Al-Shammari, A.M., Salman, M.I., 2024. Antimetastatic and antitumor activities of oncolytic NDV AMHA1 in a 3D culture model of breast cancer. Front. Mol. Biosci. 11, 1-14.

Bhutia, S.K., Mukhopadhyay, S., Sinha, N., Das, D.N., Panda, P.K., Patra, S.K., Maiti, T.K., Mandal, M., Dent, P., Wang, X.Y., Das, S.K., Sarkar, D., Fisher, P.B., 2013. Autophagy: Cancer's friend or foe? Adv. Cancer Res. 118, 61-95.

Boland, K., Flanagan, L., Prehn, J.H.M., 2013. Paracrine control of tissue regeneration and cell proliferation by Caspase-3. Cell Death Dis. 4, e725.

Bu, X., Wang, M., Zhang, Z., Zhao, Y., Li, M., Yan, Y., 2015. Autophagy is involved in recombinant Newcastle disease virus (rL-RVG)-induced cell death of stomach adenocarcinoma cells in vitro. Int. J. Oncol. 47, 679-689

Ciuffreda, L., Di Sanza, C., Incani, U.C., Milella, M., 2010. The mTOR pathway: a new target in cancer therapy. Curr. Cancer Drug Targets 10, 484-95.

Elankumaran, S., Rockemann, D., Samal, S.K., 2006. Newcastle disease virus exerts oncolysis by both intrinsic and extrinsic caspase-dependent pathways of cell death. J. Virol. 80, 7522-7534.

Forbes, S.A., Bindal, N., Bamford, S., Cole, C., Kok, C.Y., Beare, D., Jia, M., Shepherd, R., Leung, K., Menzies, A., Teague, J.W., Campbell, P.J., Stratton, M.R., Futreal, P.A., 2011. COSMIC: mining complete cancer genomes in the catalogue of somatic mutations in cancer. Nucleic Acids Res. 39, 945-950.

Ganar, K., Das, M., Sinha, S., Kumar, S., 2014. Newcastle disease virus: Current status and our understanding. Virus Res. 184, 71-81.

Ghrici, M., Zowalaty, M.E., Omar, A.R., Ideris, A., 2013. Newcastle disease virus Malaysian strain AF2240 induces apoptosis in MCF-7 human breast carcinoma cells at an early stage of the virus life cycle. Int. J. Mol. Med. 31, 525-532.

Gui, D., Cui, Z., Zhang, L., Yu, C., Yao, D., Xu, M., Chen, M., Wu, P., Li, G., Wang, L., Huang, X., 2017. Salidroside attenuates hypoxia-induced pulmonary arterial smooth muscle cell proliferation and apoptosis resistance by upregulating autophagy through the AMPK–mTOR–ULK1 pathway. BMC Pulm. Med. 17, 1-12.

Hanahan, D., 2022. Hallmarks of cancer: new dimensions. Cancer Discov. 12, 31-46.

He, K., Zheng, X., Li, M., Zhang, L., Yu, J., 2016. mTOR inhibitors induce apoptosis in colon can-

cer cells via CHOP-dependent DR5 induction on 4E-BP1 dephosphorylation. Oncogene 35, 148-157.
Jiang, K., Li, Y., Zhu, Q., Xu, J., Wang, Y., Deng, W., Liu, Q., Zhang, G., Meng, S., 2014. Pharmacological modulation of autophagy enhances Newcastle disease virus-mediated oncolysis in drug-resistant lung cancer cells. BMC Cancer 14, 1-15.
Juthani, R., Punatar, S., Mittra, I., 2024. New light on chemotherapy toxicity and its prevention. BJC Reports, 2, 41.
Kan, X., Yin, Y., Song, C., Tan, L., Qiu, X., Liao, Y., Liu, W., Meng, S., Sun, Y., Ding, C., 2021. Newcastle-disease-virus-induced ferroptosis through nutrient deprivation and ferritinophagy in tumor cells. iScience 24, 1-15.
Kim, S., Jung, B.K., Kim, J., Jeon, J.H., Jang, S.H., Kim, M., Kim, C.S., Jang, H., 2024. Newcastle disease virus harboring the PTEN gene inhibits pancreatic cancer growth by inhibiting PI3K/AKT/mTOR signaling and activating apoptosis. Mol. Ther. Oncol. 32, 200898.
Lin, D., Shen, Y., Liang, T., 2023. Oncolytic virotherapy: basic principles, recent advances and future directions. Signal Transduct. Target Ther. 8, 156.
Lopez-Knowles, E., O'Toole, S.A., McNeil, C.M., Millar, E.K., Qiu, M.R., Crea, P., Daly, R.J., Musgrove, E.A., Sutherland, R.L., 2010. PI3K pathway activation in breast cancer is associated with the basal-like phenotype and cancer-specific mortality. Int. J. Cancer 126, 1121-1131.
Meng, C., Zhou, Z., Jiang, K., Yu, S., Jia, L., Wu, Y., Liu, Y., Meng, S., Ding, C., 2012. Newcastle disease virus triggers autophagy in U251 glioma cells to enhance virus replication. Arch. Virol. 157, 1011-1018
Noor, Z., Ahmad, H., Ain, Q., Anjum, T., Malik, Z., Hussain, Z., Naseer, F., 2022. Caspase 3 and its role in the pathogenesis of cancer. Clin. Oncol. 7, 1-10.
Pathak, U., Pal, R.B., Malik, N., 2023. The viral knock: ameliorating cancer treatment with oncolytic Newcastle disease virus. Life (Basel) 13, 1-14.
Pyo, J.O., Nah, J., Jung, Y.K., 2012. Molecules and their functions in autophagy. Exp. Mol. Med. 44, 73-80.
Rakhmawati, I., Adi, A.A.A.M., Winaya, I.B.O., Sewoyo, P.S., 2022. In vivo oncolytic potency of Newcastle disease virus Gianyar-1/AK/2014 virulent strain against mice fibrosarcoma models. Rev. Electron. Vet. 23, 56-63.
Ren, S., Rehman, Z.U., Shi, M., Yang, B., Qu, Y., Yang, X.F., Shao, Q., Meng, C., Yang, Z., Gao, X., Sun, Y., Ding, C., 2019. Syncytia generated by hemagglutinin-neuraminidase and fusion proteins of virulent Newcastle disease virus induce complete autophagy by activating AMPK–mTORC1–ULK1 signaling. Vet. Microbiol. 230, 283-290.
Sewoyo, P.S., Adi, A.A.A.M., Winaya, I.B.O., Sampurna, I.P., Bramardipa, A.A.B., 2021. The Potency of Virulent Newcastle Disease Virus Tabanan-1/ARP/2017 as Virotherapy Agents in Rat Fibrosarcoma Models. J. Adv. Vet. Res. 11, 64-68.
Sewoyo, P.S., Astawa, I.N.M., Adi, A.A.A.M., Purwitasari, M.S., Hartaputera, I.N.S.T., Aisyah, S., 2024. Newcastle disease virus Tabanan-1/ARP/2017 inhibits growth of rat mammary carcinoma models. J. Adv. Vet. Res. 14, 563-566.
Sewoyo, P.S., Astawa, I.N.M., Adi, A.A.A.M., Suardana, I.B.K., Winaya, I.B.O., Berata, I.K., 2026. Newcastle disease virus Tabanan-1/ARP/2017 suppresses Ki67 and VEGF expression in rat mammary carcinoma models. Vet. Arhiv. 96, 73-81.
Wang, J., Li, M., Li, M., 2023. Newcastle disease virus LaSota strain induces apoptosis and activates the TNFα/NF-κB pathway in canine mammary carcinoma cells. Vet. Comp. Oncol. 21, 520-532.
Wang, S., Li, H., Yuan, M., Fan, H., Cai, Z., 2022. Role of AMPK in autophagy. Front. Physiol. 13, 1-11.
Ye, T., Jiang, K., Wei, L., Barr, M.P., Xu, Q., Zhang, G., Ding, C., Meng, S., Piao, H., 2018. Oncolytic Newcastle disease virus induces autophagy-dependent immunogenic cell death in lung cancer cells. Am. J. Cancer Res. 8, 1514-1527.
Yun, C.W., Lee, S.H., 2018. The roles of autophagy in cancer. Int. J. Mol. Sci. 19, 1-18.