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Original Article

The Effect of Autophagy Induction in Oncolytic Reovirus Replication in Mesenchymal Stem Cells

Shiva Maleki [1] M.Sc., Hoorieh Soleimanjahi [1*] Ph.D., Asghar Abdoli [2] Ph.D., Hesam Karimi [1] M.Sc., Razieh Sadat Banijamali [1] M.Sc., Taravat Bamdad [1] Ph.D.

[1] Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[2] Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran

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Background and Aims: Oncolytic reoviruses can infect and kill malignant cells while sparing their normal counterparts. Reoviral infection can induce or activate autophagy, even though metformin can induce autophagy. Identifying and regulating the cellular pathways important for reovirus replication and oncolysis can improve targeted-biological therapies for cancer. Here, the autophagic process was triggered via metformin, and we investigated the effect of autophagy activation on oncolytic reovirus replication in mesenchymal stem cells as primary cells and L929 cell lines.

Materials and Methods: Adipose derived mesenchymal stem cells (AD-MSCs) and L929 cells were treated with metformin and reovirus type-3 strain Dearing (T3D). Twenty-four hours after infection, the viability of AD-MSCs and L929 cells were examined by MTT assay. Also, the effect of metformin-induced autophagy in the reovirus replication in these cells was determined by real-time polymerase-chain-reaction.

Results: Our results show that treatment with metformin and reovirus reduced the viability of the cells compared to treatment with metformin or reovirus alone in both cells. Also, coadministration of metformin and reovirus significantly decreased the relative expression level of the Beclin-1 gene compared to treatment with metformin in both cells. However, the expression level of the reovirus L3 gene after treatment with metformin and reovirus in L929 cells increased significantly compared to AD-MSCs.

Conclusion: Our data suggest that metformin-induced autophagy enhances reoviral replication in AD-MSCs and L929 cells. These findings represent the role of autophagy induction in facilitating reovirus replication and contribute to a better understanding of reovirus-host interactions.

***Corresponding Author:** Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. **Tel/Fax:** +982182883561, **Email:** soleim_h@modares.ac.ir

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Introduction

Autophagy is an essential intracellular degradative process known as type II programmed cell death through a lysosomal-dependent degradation mechanism. Autophagy plays several roles in adaptation to stress conditions, cell death, and tumor suppression [1]. Beclin-1 and LC3 are connected with the initial steps of autophagosome formation and maturation during the autophagy process, respectively. Beclin-1 is an important, primary molecule that acts as a platform for autophagy establishment [2-4] to start autophagy.

Many drugs, such as metformin, are currently approved by the Food and Drug Administration (FDA) that induce autophagy [5]. Metformin is an AMP-activated protein kinase (AMPK) activator and activates autophagy via a reduction in the mammalian target of rapamycin (mTOR) signaling pathway [6].

Oncolytic viruses are novel anti-cancer therapeutics that induce an immune response and demonstrate minimal human toxicity [7]. Oncolytic reovirus has shown oncolytic potential against over 80% of human cancer cell lines. Clinical trials demonstrated that therapies based on oncolytic reovirus are safe and tolerated in patients with a wide variety of cancers [8].

Autophagy is one of the probable mechanisms of oncolysis by oncolytic reovirus. It has been suggested that oncolytic reovirus induces autophagy to kill cancer cells by exploiting p17 protein in the PI3K-Akt-mTORC1 pathway [9]. Several studies have suggested a novel vehicle system for virus delivery by mesenchymal stem cells (MSCs) to obtain optimum delivery and maximum antitumor effects of these oncolytic viruses [10, 11]. Before using MSCs as a carrier for oncolytic reovirus, the relationship between the effects of induction of autophagy on the replication of oncolytic reovirus in the MSCs should be examined. This study evaluated the effect of autophagy induction using metformin on the replication of reovirus T3D in the adipose derived mesenchymal stem cells (AD-MSCs) and L929 cell as control cell lines.

Materials and Methods

AD-MSCs cultures

The AD-MSCs were isolated from abdomen adipose tissues of C57BL/6 mice (Pasteur Institute of Iran) and were characterized and maintained as previously described [12]. The cells expressed CD29, CD90, and CD105 and were negative for CD34 and CD45. AD-MSCs were also examined for their ability to differentiate between adipocyte and osteocyte.

Cell lines and culture conditions

Murine L929 fibroblasts cell lines were provided by Dr. Soudi (Tarbiat Modares University, Iran). The cells were maintained at 37 °C with 5% CO_2 and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented by 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA).

Virus preparation

Reovirus T3D was propagated in L929 cell culture in serum-free DMEM. Briefly, L929 cells were infected at multiplicities of infection

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(MOI) of 0.1 and allowed to incubate at 37°C until a complete cytopathic effect was observed, at which point the virus was collected. Viruses were harvested by three freezes (-70 °C)-thaw cycles and clarified by low-speed centrifugation for 15 min. Large stocks of viruses are kept frozen at -70 °C in aliquots. Virus titer was determined by cell culture infectious dose 50% ($CCID_{50}$) assay as previously described [13].

Chemicals

Metformin (Sigma-Aldrich, USA) was diluted in sterile water to make the 1M stock solution and used at a concentration of 5 mM in all the experiments, except cell viability assay, in which concentrations ranging from 2.5 to 20mM were tested.

Cytotoxicity assay

Cytotoxicity was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay based on the manufacturer's protocol (Bioidea, Iran). Briefly, L929 cells were seeded into the 96-well plates. After overnight incubation at 37 °C, these cells were treated with metformin at different concentrations (2.5 to 20 mM). Three wells were remained untreated as a control. At 24 hours after incubation at 37 °C and 5% CO_2, 10 µl of MTT reagent was added to each well and incubated for four hours; the cell-free wells were considered blank controls. Then, the MTT solution was removed, and 50 µl of dimethyl sulfoxide was added to each well. The plate was maintained for 15 min at 37°C, the wells' optical density was determined at 570 nm through a spectrophotometric microplate reader.

Cell viability assay

The viability of treated AD-MSCs and L929 cells was determined using MTT assay, which evaluates the percentage of viable cells. AD-MSCs and L929 cells were seeded into a 96-well plate and treated with 5mM of metformin for 12 hours. After that, the cells were infected with reovirus T3D at MOI of 0.01. Twenty-four hours after infection, cell viability was measured by MTT assay, similar to the previous section. Experimental groups are shown in Table 1.

Table 1. Experimental groups

MRA	Metformin/Reovirus /AD-MSCs	AD-MSCs which treated with metformin and reovirus
MA	Metformin/AD-MSCs	AD-MSCs which treated with metformin
RA	Reovirus /AD-MSCs	AD-MSCs which infected with reovirus
MRL	Metformin / Reovirus / L929 cells	L929 cells which treated with metformin and reovirus
ML	Metformin / L929 cells	L929 cells treated with metformin
RL	Reovirus/L929 cells	L929 cells which infected with reovirus

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RNA extraction and real-time polymerase chain reaction (PCR)

AD-MSCs and L929 cells were seeded in 48-well plates. After 24 hours of incubation, the cells were treated with 5 mM of metformin. At 12 hours after treatment, the cells were infected with reovirus T3D at an MOI of 0.01. According to the manufacturer's protocol, total RNA was isolated from cultured cells using YTA Total RNA Purification Mini kit (Yekta tajhiz azma, Iran) 24 hours after infection. A total of 3 µl of RNA was employed to synthesize complementary DNA (cDNA) by RT series kit (BioFact™, Korea) with Random Hexamer and oligo dT primers.

Real-time PCR was performed using EvaGreen® qPCR mix plus [7] and step one plus real-time PCR system (Applied Biosystems, USA). Primer sequences were as follows: L3 (major capsid protein lambda 1) gene segment of Reovirus:

5′-CGCGTCCTCAATTTTGGGTAAAC-3′(F) 5′-CCGCCGTCTTTTGGATATGAACTA-3′(R) [12]; Beclin-1: 5′-ACAGCCCAGGCGAAACCAG-3′(F) 5′-CCTCCCCGATCAGAGTGAAGC-3′(R); HPRT1: 5′-TCCCAGCGTCGACTTAGACT-3′(F) 5′-CGAGCAAGTCTTTCAGTCC-3′(R).

RT-PCR was conducted with the following amplification conditions: 1 cycle of 95 °C for 15 min, 40 cycles of 95 °C for 15 sec, 62 °C for 20 sec and 72 °C for 30 sec. The expression revealed by each assay was normalized using the HPRT1 expression level as a control. This study was approved by the Research Ethics Committee of Tarbiat Modares University, Tehran, Iran (Code: IR.TMU.REC.1395.468).

Statistical analysis

All of the experiments in this research were performed three times in three replicates. The data are presented as mean ± standard deviation and analyzed by GraphPad Prism 7.04. statistically, significance was compared between groups using one-way ANOVA analysis of variance followed by Dennett's test for real-time PCR and Tukey's test for viability assay. Asterisks indicated groups are significantly different from each other (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$).

Results

MTT assay

L929 cells were treated with various concentrations of metformin ranging from 2.5-20 mM for 24 hours. With the increasing amount of metformin, the cell viability was decreased using MTT assay. The half-maximal (50%) inhibitory concentration (IC_{50}) values were 5 mM for the cells incubated for 24 hours, and this concentration was used in all experiments (Fig. 1).

AD-MSCs and L929 cells were measured with 5 mM of metformin for 12 hours to measure viable cells. Then, the cells were infected with reovirus T3D at MOI of 0.01. Twenty-four hours after infection, the percentage of viable cells was measured by MTT assay. As illustrated in Figure 2, the percentage of viable cells in AD-MSCs after treatment with metformin and infection with reovirus (MRA group) reduced significantly compared to MA and AD-MSCs which infected with MOI:0.01 of reovirus (RA groups). Furthermore, co-treatment of metformin and infection with reovirus in L929 cells (MRL group) significantly reduced cell viability compared to metformin

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(ML group) or reovirus infection (RL group) alone. However, at a steady-state, there was no significant difference between MRA and MRL groups.

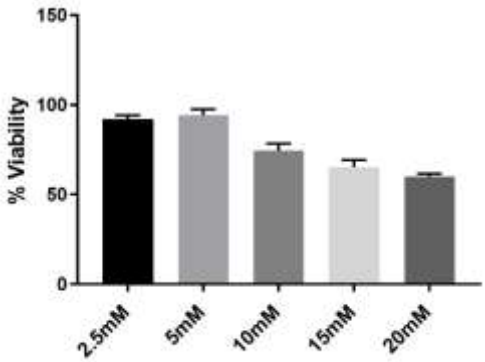

Fig. 1. IC_{50} values of metformin in L929 cells at 24 hours as determined by using the MTT assay. After being treated with different concentrations of metformin (2.5-20 mM), IC_{50} values were 5 mM for the cells incubated for 24 hours

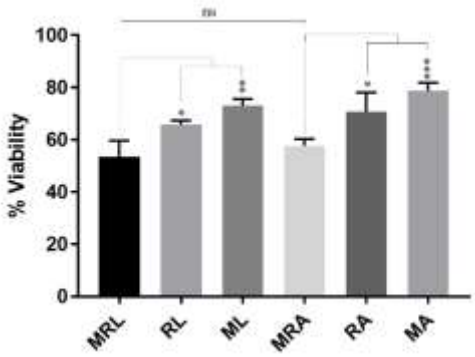

Fig. 2. The viability of AD-MSCs and L929 cells after exposure to the 5 mM of metformin and reovirus T3D infection with MOI=0.01 as determined using MTT assay. MA group= AD-MSCs treated with 5 mM of metformin; RA group= AD-MSCs which infected with MOI:0.01 of reovirus; MRA group= AD-MSCs treated with 5 mM of metformin and infected with MOI:1 of reovirus; ML group= L929 cells treated with 5 mM of metformin; RL group= L929 cells infected with MOI:0.01 of reovirus and MRL group= L929 cells treated with 5 mM of metformin and infected with MOI:1 of reovirus

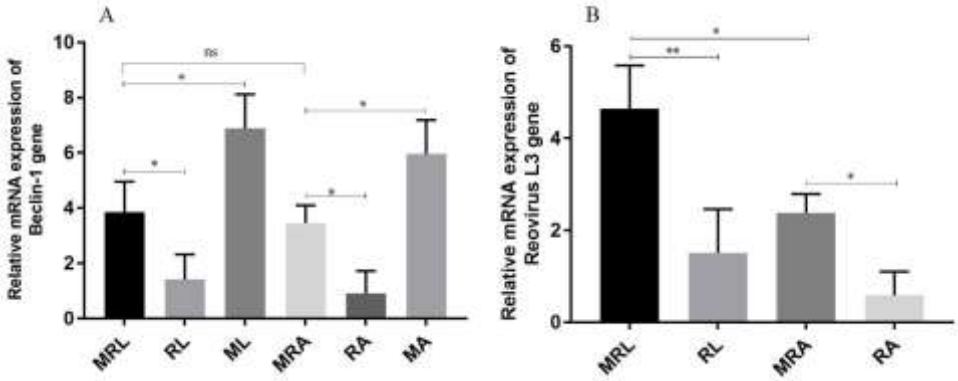

Fig. 3 (A, B). The gene expression ratio of Beclin-1 gene (A) and reovirus L3 gene (B) in AD-MSCs and L929 cells after treatment with 5 mM of metformin and infection with MOI:0.01 of reovirus T3D compared to the metformin or reovirus infection alone groups MA group= AD-MSCs which treated with 5 mM of metformin; RA group= AD-MSCs infected with MOI:0.01 of reovirus; MRA group= AD-MSCs which treated with 5 mM of metformin and infected with MOI:1 of reovirus; ML group= L929 cells treated with 5 mM of metformin; RL group= L929 cells infected with MOI:0.01 of reovirus and MRL group= L929 cells treated with 5 mM of metformin and infected with MOI:1 of reovirus.

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Real-time PCR analysis

The changes in the relative expression level of Beclin-1 and reovirus L3 gene were observed to determine the effect of metformin and reovirus infection on induction of autophagy and its relation to reovirus replication. As shown in Figure 3A, in the MRA group, the relative expression level of the Beclin-1 gene increased significantly compared to the RA group. However, the relative expression level of the Beclin-1 gene in the MRA group decreased significantly compared to the AD-MSCs treated with 5 mM of metformin (MA group). In the MRL group, co-treatment of metformin and reovirus infection significantly increased the relative expression level of the Beclin-1 gene compared to the RL group. On the other hand, the relative expression level of the Beclin-1 gene in the MRL group decreased significantly compared to the ML group. However, at a steady-state, there was no significant difference between their effect in primary and line cells (MRA and MRL groups).

As demonstrated in Figure 3B, the relative expression level of the reovirus L3 gene in AD-MSCs after treatment with metformin and infection with reovirus (MRA group) increased significantly compared with reovirus infection alone (RA group). Also, in the MRL group, co-treatment with metformin and reovirus infection significantly increased the relative expression level of the reovirus L3 gene compared to infection with reovirus. However, the relative expression level of the reovirus L3 gene in the MRL group increased significantly compared to the MRA group.

Discussion

Given that cancer is a complex multistage process and that autophagy performs its effects in different ways, its role in tumor treatment with virotherapy should be evaluated [1]. Autophagy is an essential, conserved process by which cytoplasmic components are degraded by lysosomes [14]. Beclin-1, the mammalian orthologue of yeast Atg6, has a central role in autophagy initiation [2]. In this way, metformin, an anti-diabetic drug, can trigger autophagy [15]. However, other studies demonstrated that reovirus induces autophagy in tumor cells to destroy them [9]. However, to enhance the quality of treatment with an oncolytic virus, many studies suggested using MSCs as a vehicle [16]. Also, Banijamali et al. [17] suggested that AD-MSCs can be used as effective carrier cells candidates for reovirus T3D to maximize their anti-cancer cell activity.

Nevertheless, a better understanding of the reovirus-host interaction is crucial to improving therapeutic strategies for oncolytic virotherapy. This study demonstrates the viability of AD-MSCs and L929 cells after treatment with metformin and reovirus infection. Furthermore, the relationship between the autophagy induction with metformin in the replication of reovirus was evaluated. The results of this study revealed that combined treatment of metformin and reovirus infection in both AD-MSCs and L929 cells (MRA and MRL groups) significantly increased cell death compared to treatment with metformin (MA and ML groups) or reovirus infection (RA and RL groups) alone.

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So, treatment with metformin and reovirus T3D infection promoted the cell death of AD-MSCs as a primary cell and L929 line cells. Cell death may occur through active mechanisms, for example, cellular suicide or active or programmed cell death. At a steady-state, there was no significant difference between results from AD-MSCs and L929 cells. Some reports have shown that metformin treatment can induce apoptosis [18] and autophagy [19] in some types of cancer. Metformin exhibited significant synergy with chemotherapeutic drugs models via broad-spectrum action mechanisms. The combination of metformin and sorafenib prevents proliferation and triggers autophagy of hepatocellular carcinoma through targeting the mTOR pathway [20]. Nazim et al. [21] found co-treatment of metformin with tumor necrosis factor-related apoptosis-inducing ligand significantly induced cell death of human lung adenocarcinoma A549 cells. Yang et al. studied the efficiency of PEGylated liposomes comprising metformin and epirubicin against $CD133^{+}$ cancer stem-like cells [22]. Their results exhibited that liposomes-encapsulated metformin and epirubicin induce cell death compared to the metformin and epirubicin alone. Buzzai and colleagues [23] pointed that metformin induces autophagy in HCT116 colon cancer cells.

Beclin-1 has a key role in autophagy because it is involved in the triggering of autophagosomes formation [24]. Our real-time PCR results demonstrated that treatment with metformin alone significantly increased the relative expression level of the Beclin-1 in AD-MSCs and L929 cells (MA and ML groups) compared with MRA and MRL groups. In contrast, co-treatment of metformin with reovirus (MRA and MRL groups) significantly increased the relative expression level of the Beclin-1 compared to treatment with reovirus infection alone (RA and RL groups). However, at a steady-state, there was no significant difference between results from AD-MSCs and L929 cells. It seems that coadministration of the metformin and reovirus has a synergistic effect on the increase of autophagy compared with virotherapy alone, which mutually reinforces the virus replication.

Parallel with this study, Xie and colleagues [25] have shown that metformin dramatically enhanced Beclin-1 protein expression in OVE26 mice. On the other hand, it has been demonstrated that viruses have evolved ways to suppress or induce the autophagy machinery to facilitate their replication and survival [26].

This *in-vitro* study examined metformin-induced autophagy in AD-MSCs and L929 cells at the early stage of infection with reovirus T3D. Our data suggest that autophagy induced via metformin enhances viral replication in AD-MSCs and L929 cells at the early stage of infection. Nevertheless, the reovirus L3 gene expression level in the MRL group increased significantly compared to the MRA group because L929 cells are a susceptible host cell line for reovirus T3D. Niu and colleagues [27] reported that avian reovirus induces autophagy, which benefits its replication and dissemination in chicken tissues. Similarly, Chi et al. [28] reported that autophagy induction through rapamycin treatment increased avian reoviral protein synthesis and viral yield.

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Conclusions

Overall, autophagy can be stimulated by metformin and reovirus T3D infection and plays a positive role in reovirus T3D replication. Also, the oncolytic effect of reovirus was increased by simultaneous application of metformin. Still, the interplay between autophagy and cell death, leading to oncolysis in reovirus T3D infection, needs further study to understand the crosstalk between autophagy and reovirus oncolytic activity deeply.

Conflict of Interest

The authors declared no conflict of interest.

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References

[1]. Yun C, Lee S. The roles of autophagy in cancer. Int J Mol Sci. 2018; 19(11): 3466.
[2]. Kang R, Zeh H, Lotze M, Tang D. The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differentia 2011; 18(4): 571.
[3]. Yu L, Chen Y, Tooze SA. Autophagy pathway: cellular and molecular mechanisms. Autophagy 2018; 14(2): 207-15.
[4]. Feizi N, Mehrbod P, Romani B, Soleimanjahi H, Bamdad T, Feizi A, et al. Autophagy induction regulates influenza virus replication in a time-dependent manner. J Med Microbiol. 2017; 66(4): 536-41.
[5]. Levine B, Packer M, Codogno P. Development of autophagy inducers in clinical medicine. J Clinic Investig. 2015; 125(1): 14-24.
[6]. Tsai HH, Lai HY, Chen YC, Li CF, Huang HS, Liu H, et al. Metformin promotes apoptosis in hepatocellular carcinoma through the CEBPD-induced autophagy pathway. Oncotarget 2017; 8(8): 13832.
[7]. Phillips MB, Stuart JD, Stewart RMR, Berry JT, Mainou BA, Boehme KW. Current understanding of reovirus oncolysis mechanisms. Oncolytic Virotherapy 2018; 7(1): 53.
[8]. Mostafa A, Meyers D, Thirukkumaran C, Liu P, Gratton K, Spurrell J, et al. Oncolytic reovirus and immune checkpoint inhibition as a novel immunotherapeutic strategy for breast cancer. Cancers 2018; 10(6): 205.
[9]. Chiu HC, Richart S, Lin FY, Hsu WL, Liu HJ. The interplay of reovirus with autophagy. BioMed Research International. 2014; 1: 1-10.
[10]. Komarova S, Kawakami Y, Stoff-Khalili MA, Curiel DT, Pereboeva L. Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. Mol Cancer Therapeut. 2006; 5(3): 755-66.
[11]. Muhammad T, Sakhawat A, Khan AA, Ma L, Gjerset RA, Huang Y. Mesenchymal stem cell-mediated delivery of therapeutic adenoviral vectors to prostate cancer. Stem Cell Res Therapy 2019; 10(1): 190.
[12]. Banijamali RS, Soleimanjahi H, Soudi S, Karimi H, Abdoli A, Khorrami SS, et al. Kinetics of oncolytic reovirus T3D Replication and growth pattern in mesenchymal stem cells. Cell J. 2020; 22(3): 283-92.
[13]. Banijamali RS, Soleimanjahi H, Soudi S, Karimi H. The effect of oncolytic reovirus infection on nitric oxide secretion and induction of apoptosis in adipose tissue-derived mesenchymal stem cells. Iranian Journal of Medical Microbiology 2018; 12(3): 218-29.
[14]. Soleimanjahi H, Abdoli A. Role of chaperone mediated autophagy in viral infections. In: Asea AAA, Kaur P, editors. Chaperokine Activity of Heat Shock Proteins. Cham: Springer International Publishing; 2019. p. 147-54.
[15]. De Santi M, Baldelli G, Diotallevi A, Galluzzi L, Schiavano GF, Brandi G. Metformin prevents cell tumorigenesis through autophagy-related cell death. Scientific Rep. 2019; 9(1): 66.
[16]. Kazimirsky G, Jiang W, Slavin S, Zivav A, Brodie C. Mesenchymal stem cells enhance the oncolytic effect of Newcastle disease virus in glioma cells and glioma stem cells via the secretion of TRAIL. Stem Cell Res Therapy 2016; 7(1): 149.
[17]. Banijamali RS, Soleimanjahi H, Soudi S, Karimi, H. Mesenchymal stem cells support delivery and boost the efficacy of oncolytic reoviruses in TC-1 tumor cells. Journal of Cellular Biochemistry 2021; 122(10): 1360-375.
[18]. Liu B, Fan Z, Edgerton SM, Deng XS, Alimova IN, Lind SE, et al. Metformin induces unique biological and molecular responses in

[DOI: 10.18502/ijml.v8i4.8098]

triple negative breast cancer cells. Cell Cycle 2009; 8(13): 2031-2040.

[19]. Shi W, Xiao D, Wang L, Dong L, Yan Z, Shen Z, et al. Therapeutic metformin/AMPK activation blocked lymphoma cell growth via inhibition of mTOR pathway and induction of autophagy. Cell Death Dis. 2012; 3(3): 275.

[20]. Ling S, Song L, Fan N, Feng T, Liu L, Yang X, et al. Combination of metformin and sorafenib suppresses proliferation and induces autophagy of hepatocellular carcinoma via targeting the mTOR pathway. Int J Oncol. 2017; 50(1): 297-309.

[21]. Nazim UM, Moon JH, Lee JH, Lee YJ, Seol JW, Eo SK, et al. Activation of autophagy flux by metformin downregulates cellular FLICE-like inhibitory protein and enhances TRAIL-induced apoptosis. Oncotarget 2016; 7(17): 23468.

[22]. Yang Q, Zhang T, Wang C, Jiao J, Li J, Deng Y. Coencapsulation of epirubicin and metformin in PEGylated liposomes inhibits the recurrence of murine sarcoma S180 existing CD133+ cancer stem-like cells. Euro J Pharmaceut Biopharm. 2014; 88(3): 737-45.

[23]. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, et al. Systemic treatment with the anti-diabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res. 2007; 67(14): 6745-752.

[24]. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999; 402(6762): 672.

[25]. Xie Z, Lau K, Eby B, Lozano P, He C, Pennington B, et al. Improvement of cardiac functions by chronic metformin treatment is associated with enhanced cardiac autophagy in diabetic OVE26 mice. Diabetes 2011; 60(6): 1770-778.

[26]. Lennemann NJ, Coyne CB. Catch me if you can: the link between autophagy and viruses. PLoS Pathogens 2015; 11(3): 1004685.

[27]. Niu X, Zhang C, Wang Y, Guo M, Ruan B, Wang X, et al. Autophagy induced by avian reovirus enhances viral replication in chickens at the early stage of infection. BMC Veterin Res. 2019; 15(1): 173.

[28]. Chi PI, Huang WR, Lai IH, Cheng CY, Liu HJ. The p17 nonstructural protein of avian reovirus triggers autophagy enhancing virus replication via activation of phosphatase and tensin deleted on chromosome 10 (PTEN) and AMP-activated protein kinase (AMPK), as well as dsRNA-dependent protein kinase (PKR)/eIF2α signaling pathways. J Biol Chem. 2013; 288(5): 3571-584.

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