Induction of Apoptosis and Autophagy Using Ectopic DSCR1 Expression in Breast Cancer Cells

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Abstract: Down syndrome critical region 1 gene (DSCR1) is an anti-angiogenesis gene that inhibits the growth of tumor cells. In this study, the role of autophagy and apoptosis in DSCR1-induced cytotoxicity were investigated in MDA-MB-468 breast cancer cells. Lentivirus vector harboring DSCR1 (LV-DSCR1+) was constructed in HEK 293 cells and the optimal dosage of lentivirus vector for infection was determined by the MTT assay. After infection of cells using LV-DSCR1+, acridine orange and ethidium bromide staining was performed to investigation of apoptosis and autophagy. Expression of DSCR1 and marker genes for angiogenesis (VEGF), apoptosis (Bax and Bcl2) and autophagy (LC3 and Beclin) were determined by Real time PCR. The cellular morphological changes related to apoptosis and autophagy was happened after 48 hours of viral infection. Fragmented bright orange nucleuses and vacuoles were observed due to the cell apoptosis and autophagy after acridine orange and ethidium bromide staining. Upregulation of Bax, Lc3, DSCR1 and Beclin1 and downregulation of Bcl2 and VEGF was detected due to treatment with LV-DSCR1+. These results demonstrated that LV-DSCR1+ can induce apoptosis and autophagy, therefore suggesting that it may serves as an efficient tool to breast cancer treatment.

Keywords: Breast cancer, DSCR1, lentiviruses, apoptosis, autophagy.

1 Introduction
Breast cancer is one of the common cancers in women. Despite of conventional therapies and novel progressed techniques in diagnosis and therapy, breast cancer still a devastating disease worldwide [Hanahan and Weinberg (2011); Ravindra, Tiwari, Sharma et al. (2009); Majumdar (2000)]. Therefore it is necessary to searching of new more effective therapeutics [Stoff, Dall and Curiel (2006)]. Gene therapy is the leading way to treat cancer in many investigational models. This method is based upon the recognition of tumor suppressor or suicide proteins encoding genes and transferring them into tumor cells by means of a genetic vector. Viruses are mostly chosen as vehicles for gene therapy because they have evolved very effective mechanisms of gene transfer and expression [Ring and Blair (2000)].

Defective levels of autophagy or apoptosis result in uncontrolled cell proliferation, such as cancer. Cancers are happened mostly due to their defective activities and are important
to be evaluated for development of new anticancer agents. Angiogenesis has vital role to tumor progression therefore it can be a significant target for anticancer treatment [Gasparini (1999); Miller, Trigo, Wheeler et al. (2005)]. Vascular endothelial growth factor-A (VEGFA; also referred to as VEGF) is one of the critical factors for solid tumor growth through stimulating of angiogenesis [Jain (2005)]. It is suggested that the reduction occurrence in many cancer types in Down syndrome patients can be due to the high expression of some genes on chromosome 21 such as Down syndrome critical region-1 (DSCR1) encoding a protein which can suppress (VEGF)-mediated angiogenic signaling via the calcineurin pathway [Baek, Zaslavsky, Lynch et al. (2009)]. The DSCR1 (also known as RCAN1) gene consists of seven exons, alternatively transcribed or spliced of exons 1-4 produces different mRNA isoforms [Davies, Ermak, Rothermel et al. (2007)]. Calcineurin-NFAT signaling activates expression of RCAN1-4 isoform, in response to different stimuli such as VEGF [Hesser, Liang, Camenisch et al. (2004); Minami, Horiuchi, Miura et al. (2004); Yang (2000)]. The calcineurin and nuclear factor of activated T cells (NFAT) is the principal mediator of VEGF signaling in endothelial cells pathway [Hesser, Liang, Camenisch et al. (2004); Minami, Horiuchi, Miura et al. (2004); Minami, Jiang, Schadler et al. (2013)]. VEGF activation on endothelial cells cause to intracellular calcium increasing and the calcium regulated Ser/Thr phosphatase calcineurin activation. Calcineurin dephosphorylates the NFAT transcription factors family and in consequence permitting their nuclear entry and induction of DSCR1 as its endogenous inhibitor. A negative feedback loop as a result of Upregulation of DSCR1 inhibits calcineurin activity [Minami, Horiuchi, Miura et al. (2004); Minami, Miura, Aird et al. (2006)].

Lentiviral vectors (LVs) can deliver large cDNAs to dividing and nondividing target cells [Kafri, van Praag, Gage et al. (2000)]. These vectors, such as those based on human immunodeficiency virus 1 (HIV-1), transduce a variety of cell types, including embryonic and adult stem cells, and have been suggested as candidate vectors for both in vivo and ex vivo gene therapy applications [Hamaguchi, Woods, Panagopoulos et al. (2000)]. However, integration of viral genome into host cell genome may induce some adverse effects, such as insertional mutagenesis; this has been highlighted by the induction of malignancy in mouse models [Li, Düllmann, Schiedlmeier et al. (2000)] and development of leukemia in five patients in two clinical gene therapy trials [Hacein-Bey-Abina, Garrigue, Wang et al. (2008)]. Improving safety and efficiency of LV has been achieved, for example, by modifications of packaging cassettes on the virus integrase gene or on other regions of virus genome. This virus, which is called the nonintegrated LV, cannot integrate into the host genome [Cockrell and Kafri (2007)].

In this paper we studied anti-cancer effect of DSCR1 to investigate this new approach as a new strategy for human cancer therapy. DSCR1 induced programmed cell death in MDA-MB-468 which is a triple negative breast cancer cell line. We studied the expression changes in apoptotic, autophagic and angiogenic genes in cells infected with lentiviruses and compared the results with controls. The results of this investigation provide scientific evidence for the application of LV-DSCR1+ in breast cancer therapy.
2 Material and methods

2.1 Cancer and normal cell lines

MDA-MB-468 breast cancer cells were cultured in DMEM, both containing 10% inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO₂ in air. The cells were grown to confluence and sub-cultured every three to four days.

2.2 Construction of the LV harboring DSCR1

HEK293 cells were cultured in 75 cm² plastic culture flask in high glucose DMEM and were prepared to a level of confluence. The transfer construct EX-S0552-LV-105-B harboring DSCR1 (GeneCopia) was co-transfected with the enveloped plasmid pMD2G (InvivoGen) and the packaging plasmid pLV-HELP (InvivoGen) into HEK 293T cell culture. The culture medium was changed 14 h after transfection. One day after medium change, active lentiviral vectors were released in culture medium. Culture medium was removed and viral vectors become concentrated with MILLIPORE falcons and kept in-80°C for further usage [Niki, Boroujeni and Aleyasin (2014)].

2.3 Confirmation of virus construction by analysis of DSCR1 expression in HEK 293

Expression of DSCR1 gene was determined in transduced and untransduced HEK cells in 72 h of cell culture in comparison to β-actin gene which was used as housekeeping B acting gene using quantitative RT-PCR [Moriscot, de Fraipont, Richard et al. (2005); Talebi, Aleyasin, Soleimani et al. (2012)]. Total RNA was extracted from all triplicate groups cell culture using RNA X PLUS (CinnaGen Co.). The cDNA was prepared using cDNA RT Kit (Fermentas). RT-PCR was performed using AccuPower® 2X Greenstar qPCR Master Mix Kit (Bioneer) in Rotor-Gene™ 6000 (Corbett) thermal cycler. Primer sequences and their annealing temperature and products length are shown in (Tab. 1). The identity of PCR products was confirmed by electrophoresis and sequencing. Their relative gene expression data were analyzed using 2⁻∆∆Ct method [Schmittgen and Livak (2008)].

2.4 MTT viability test on MDA-MB-468 cells infected by LV-DSCR1

The MTT assay was performed on MDA-MB-468 cells to determine the effect of LV-DSCR1. Cells were plated in triplicates into 96-well tissue culture plates at a concentration of 5×10³ cells per well in 100 μL of culture media. After 72 h the infected and non-infected (PBS was added instead of virus as a control) MDA-MB-468 culture mediums were replaced with a volume of 100 μL Ham’s F12 containing 10% tetrazolium dye 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) solution in the 96-well plates and incubated for 3 h at 37°C. The medium was changed with 100 μL DMSO solution and incubated for 10 minutes at room temperature to develop the purple color. The optical density (OD) of DMSO solution was measured at 580 nm to determine the relative cell viability [Niki Boroujeni and Aleyasin (2014)].

2.5 Transduction of MDA-MB-468 with LV-DSCR1

Cells (2×10⁵ cells/mL) were cultured in a 25 cm² plastic culture flask in appropriate medium and 10% FBS for 24 h. Concentrated LV-DSCR1 was added to cultures and
incubated for 12 h. The viral transduction was repeated twice to obtain better DSCR1 expression in cells. The exact experiment was done for NDV and total mRNA was isolated from cells for quantitative analysis.

2.6 Investigation of apoptotic and autophagic effect of DSCR1 on MDA-MB-468 by Acridine orange and ethidium bromide staining

Cells were plated in two separate 12-well tissue culture plate at a concentration of 106 cells per well in 500 µl of culture media. After 72 h the infected and non-infected (PBS was added instead of virus as a control) and cytopathic effect observation, Cells were stained with Acridine orange/Ethidium Bromide (ROCHE) mixture and apoptotic cells were investigated with fluorescent microscope. Cells were stained with Acridine orange (ROCHE) and autophagic cells were investigated with fluorescent microscope.

2.7 Analysis of angiogenesis, apoptotic and autophagic marker genes expression in cancer and normal cells infected by LV-DSCR1, by real-time reverse transcription-polymerase chain reaction

Expression of angiogenesis marker genes consisted of VEGF and DSCR1 genes, apoptosis marker genes consisted of Bax and Bcl2 genes and autophagic marker genes consisted of LC3 and Beclin were determined in infected and non-infected cell cultures in comparison to β-actin gene which was used as housekeeping gene using quantitative RT-PCR [Moriscot, de Fraipont, Richard et al. (2005); Talebi, Aleyasin, Soleimani et al. (2012)]. Total mRNA was extracted from all triplicate groups cell culture using RNA X PLUS (CinnaGen Co). cDNA was prepared using cDNA RT Kit (Fermentas). RT-PCR was performed using AccuPower® 2X Greenstar qPCR Master Mix Kit (Bioneer) in Rotor-GeneTM 6000 (Corbett) thermal cycler. Primer sequences and their annealing temperature and products length are shown in Tab. 1. The identity of PCR products was confirmed by electrophoresis and sequencing. Their relative gene expression data were analyzed using 2^-ΔΔCt method [Schmittgen and Livak (2008)].

Table 1: Primer sequences used in real-time RT-PCR experiments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Size (bp)</th>
<th>Strand</th>
<th>Sequence (5’→3’)</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSCR1</td>
<td>140</td>
<td>F</td>
<td>AACAAGTGGAAGATGCGAC</td>
<td>60</td>
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<tr>
<td></td>
<td></td>
<td>R</td>
<td>ATCACTCTCACATACATGGAC</td>
<td>60</td>
</tr>
<tr>
<td>VEGF</td>
<td>179</td>
<td>F</td>
<td>AACTTTCTGCTGTGTTGGTG</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>ATGTCCACCAGGGTCTGATT</td>
<td>60</td>
</tr>
<tr>
<td>Bax</td>
<td>140</td>
<td>F</td>
<td>CACGATGTTTCTGACGGCA</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>GAAGTCCAATGTCCAGCCA</td>
<td>60</td>
</tr>
<tr>
<td>Bcl2</td>
<td>150</td>
<td>F</td>
<td>ATTGTGGCCTTCTTTTGATTCG</td>
<td>60</td>
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<tr>
<td></td>
<td></td>
<td>R</td>
<td>ATCCACCTCCGTTATCCT</td>
<td>60</td>
</tr>
<tr>
<td>β-actin</td>
<td>161</td>
<td>F</td>
<td>GAGACCCTCAACACCCCCAGCC</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>AGACGGCAGATGGCAGGCC</td>
<td>60</td>
</tr>
<tr>
<td>Beclin</td>
<td>150</td>
<td>F</td>
<td>ATGCAAGGTGAGCTCGTTGTG</td>
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<tr>
<td></td>
<td></td>
<td>R</td>
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<td>AACCGCATTTGCCATCAG</td>
<td>60</td>
</tr>
<tr>
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<td>R</td>
<td>GACCTCCAGCAGTTTACAG</td>
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</tr>
</tbody>
</table>
2.8 Investigation of apoptosis induction in cultured cells after viral infection using flowcytometry

Induction of apoptosis in MDA-MB-468 cell line was detected using annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Biolegend, San Diego, CA). According to the manufacturer’s instruction, Cancer and normal cells were plated in 6-well tissue culture plate at a concentration of 100000 cells per well in 2 ml of culture media. Cells were infected by LV-DSCR1. After 72 h, apoptosis were investigated in the infected and non-infected cells were harvested and washed with cold PBS buffer, pelleted and resuspended in kit binding buffer. They were treated with annexin V-FITC conjugate and incubated in dark place at RT for 15 min. After antibody conjugation and performing PI staining (5 μg/ml) cells were analyzed for induction of apoptosis by flow cytometry equipment (Accuri® C6 Flow Cytometer, AnnArbor, MI) within 1 h. The flow cytometry data obtained from a minimum of 10000 cells per sample was analysed using BD Accuri C6 Flow software.

2.9 Statistical analysis

For gene expression comparison among infected and non-infected cells, statistical analysis was performed by one-way analysis of variance using SPSS version 12.0 (SPSS, Chicago, MI). In all statistical analyses, \( p < 0.05 \) was statistically judged significant and all \( p \) values were two-sided.

3 Results

3.1 Production of LV- DSCR1 in HEK 293 cells

The actual LV-DSCR1 vector was amplified and packaged in HEK 293 cells. These cells were co-transfected by three plasmids. The sign of viral production was an early color change in the medium from red to yellow, compared to non-transfected HEK 293.

Figure 1: The MTT assay was performed to evaluate lentiviral cytotoxicity in MDA-MB-468 cells. These Cells were treated with different volumes of viral vector for 48 h. The viability of untreated cells was considered 92%. The experiments were performed in triplicate and data is presented as the mean±SD from three independent experiments.
Results were analyzed with student’s t-test. Increasing the volume of viruses more than 0.8 μL/15000 cells resulted in significant decrease in viability and optical density (OD) of the cells. In continuous volumes lower than 0.8 μL were used at later stages.

### 3.2 MTT viability test on MDA-MB-468 cells infected by lentiviruses

The optimum volumes of LV-DSCR1 were obtained in MTT test as 0.8 μL/15000 cells. Increasing the volume of viruses more than 0.8 μL/15000 cells resulted in significant decrease in viability and optical density (OD) of the cells (Fig. 1). In continuous volumes lower than 0.8 μL were used at later stages.

### 3.3 Morphological changes of breast cancer cells after viral infection

In infected cells, the large membranous vacuoles were detected in the cytoplasm which is a characteristic feature of cells undergoing autophagy (Fig. 2A). Blebbing and cell shrinkage were detected among infected cells after viral infection which is a characteristic feature of cells undergoing apoptosis (Fig. 2B).

![Figure 2: Ectopic expression of DSCR1 reduced cell viability and induced autophagy and apoptosis in MDA-MB-468 breast cancer cells. A) Micrographs represent vesicular organelles (autophagy) and B) Blebbing and cell shrinkage (apoptosis) after infection. C) Non-infected cells do not show any signs of apoptosis and autophagy.](image)

### 3.4 Apoptosis and autophagy investigation using acridine orange and ethidium bromide staining

Acridine orange staining was used to confirm autophagy cell death after viral infection. As shown in Fig. 3A, LV-DSCR1 could induces autophagy in MDA-MB-468 cell line. Moreover, acridine orange and ethidium bromide staining were used to detect other
Induction of Apoptosis and Autophagy Using Ectopic Dscr1 Expression

modes of cell death induced by viral infection. As shown in Fig. 3B, LV-DSCR1 could also induce apoptosis.

![Figure 3: Autophagy and apoptosis induced after infection. Apoptosis and autophagy were investigated using acridine orange and ethidium bromide staining. A) The presence of orange granules in the cell cytoplasm is a sign of autophagy that induced after infection. B) Apoptotic cells were marked with concentrated and localized orange nuclear ethidium bromide staining. C) Non-infected cells do not show any signs of apoptosis and autophagy.]

3.5 Analysis of apoptotic marker gene expression in MDA-MB-468 after lentiviral infection

The lentiviral infection of MDA-MB-468 for analysis of apoptotic were performed using culture media consisting of high glucose DMEM, 10% FBS and 0.1% penicillin/streptomycin. Expression of apoptotic marker genes consist of BAX and BCL2 studied in MDA-MB-468 by quantitative RT-PCR. RNAs of cancer and normal cells were isolated in the 72 h after viral infection and cDNAs constructed. In infected cancer cells, BAX expression was increased and BCL2 was decreased in comparison with the non-infected cells after 72 h. In normal cells, the expression of apoptotic-related genes (Bax and Bcl2) showed no significant changes compared to control cells (Fig. 4A).

3.6 Analysis of angiogenesis marker gene expression in MDA-MB-468 after virus infection

Expression changes of angiogenic markers (DSCR1 and VEGF) were investigated using quantitative RT-PCR after 72 h of treatment. DSCR1 Expression was increased in infected cells compared to the non-infected cells after 72 h of infection. On the other hand, expression of VEGF was decreased compared to the non-infected one.
To determine the DSCR1 and VEGF gene expressions in infected and non-infected cells, RNA was extracted and quantitative RT-PCR was done. DSCR1 expression was increased after 72 h compared to the non-infected one. On the other hand, expression of VEGF was decreased compared to the non-infected one (Fig. 4A).

3.7 Analysis of autophagic marker genes expression in MDA-MB-468 after lentiviral infection

The lentiviral infection of cancer and normal cells were performed. Expression of autophagic marker genes consist of LC3 and Beclin studied in MDA-MB-468 by quantitative RT-PCR. In infected cancer cells, LC3 and Beclin expression was increased in comparison with the non-infected cells after 72 h. In normal cells, the expression of autophagy-related genes showed no significant changes compared to non-infected cells (Fig. 4B).

Figure 4: Apoptotic, angiogenic and autophagic marker genes expression in infected MDA-MB-468 cells in comparison to the non-infected MDA-MB-468 cells through application of the real time RT-PCR. B-Actin gene amplification was tested in infected and non-infected RPE and MCF7 cells as an internal real time RT-PCR positive control. A) Relative gene expressions of the apoptotic marker genes consisting of the Bax and Bcl2 and angiogenic marker genes consisting of the VEGF and DSCR1 in the infected cancer cells compared to the non-infected cancer cells. B) Relative gene expressions of the autophagic marker genes consisting of the Lc3 and Beclin in the infected cancer cells compared to the non-infected cancer cells. All PCR products were verified by electrophoresis in addition to sequencing and relative gene expression data were analyzed using $2^{\Delta\Delta C_t}$ method

3.8 Investigation of apoptosis in MDA-MB-468 after lentiviral infection using flowcytometry

Infection of cancer and normal cells were performed by lentiviral vectors. The percentage of apoptotic cells was determined with anti-annexin antibodies. In infected cancer cells, the percentage of apoptotic cells was increased in comparison with the non-infected cells.
after 72 h. In normal cells, the percentage of apoptotic cells showed no significant changes compared to non-infected cells (Fig. 5).

**Figure 5:** Investigation of apoptosis by flowcytometry assay using Annexin V-FITC/PI. A) Ectopic DSCR1 expression induces apoptosis in infected cancer cells in comparison to B) non-infected cancer cells

4 Discussion
Cancer is the major cause of mortality in human. In spite of the present great progress in cancer treatment, however cancer has remained a lethal disease worldwide. One of the hallmarks of breast cancer is resistance of tumor cells to cell death. Therefore, remedies based on the induction of programmed cell death attract an increased attention for cancer treatment. Autophagy and apoptosis are two forms of programmed cell death. Normally, autophagy and apoptosis have a tumor-suppressor role. Consequently, defective levels of either autophagy or apoptosis results in uncontrolled cell proliferation, such as cancer. Cancers are happened mostly due to their defective activities and are important to be evaluated for development of new anti-cancer agents. Hence, in this study, we investigated the effect of ectopic expression of DSCR1 on MDA-MB-468 as a triple negative breast cancer cell line. This study showed a significant cytotoxic effect of DSCR1 over expression on breast cancer cells that was mediated via two mechanisms including apoptosis and autophagy.

Treatment of breast cancer has been commonly performed by frequently tumor surgery, chemotherapy or radiotherapy. Some problems such as difficult surgery and chemoresistance limit the cancer treatment. Considering the lethality, limited efficacy, and toxicities of the commonly used treatments strategies, there is large demand for the use of effective and combined therapy to suppress such a complex and noxious disease [Ravindra, Tiwari, Sharma et al. (2009)]. Gene therapy is an approach is applying for the destruction of tumor cells. In this investigation DSCR1 gene were used as an anti-angiogenic factor for cancer inhibition.

DSCR1 play its anti-angiogenesis role due to interruption in angiogenesis signaling pathways by down regulating VEGF gene expression. Baek et al. [Baek, Zaslavsky,
Lynch et al. (2009)] showed that a transgenic copy of DSCR1 is sufficient to confer significant suppression of tumor growth in mice by dampening VEGF-calcineurin signaling. VEGF-mediated angiogenesis is central to tumor progression and has become a therapeutic target for anticancer treatment [Sunshine, Dallabrida, Durand et al. (2012)]. Minami et al demonstrated that overexpression of DSCR1 inhibited lung metastases in mouse models [Minami, Miura, Aird et al. (2006)]. DSCR1 is directly blocking calcineurin phosphatase function and deletion of DSCR1 in a transgenic mouse model leads to hyperactivation of calcineurin-NFAT signaling [Ryeom, Greenwald, Sharpe et al. (2003)]. In this study for the first time DSCR1 gene was transferred to MDA-MB-468 cancer cells using lentiviral vectors. After cell infection the increasing in the DSCR1 expression and the VEGF reduction were observed by real time RT-PCR. Expression of angiogenic genes including VEGF was decreased to the rate of 0.384 after infection, comparing to non-infected cells. Decreasing of VEGF expression caused to the reduction in angiogenesis that is effective in tumor growth inhibition and cancer treatment.

Lentiviral vectors can deliver transgenes to a wide variety of dividing and nondividing cells and maintain stable long-term transgene expression [An, Wersto, Metzger et al. (2000)]. However, integration of a provirus into host chromatin has induced some adverse issues, such as insertional mutagenesis and induction of malignancy in human and mouse models [Hamaguchi, Woods, Panagopoulos et al. (2000); Li, Düllmann, Schiedlmeier et al. (2002)]. Development of lentiviral vectors to deliver transgenes has provided adequate safety and efficiency in clinical applications due to modifications in the packaging cassette that have limited the potential risk for insertional mutagenesis and replication competent of lentiviruses [Cockrell and Kafri (2007)].

This is the first study to investigate the application of lentiviruses harboring DSCR1 in inhibition of angiogenesis in breast cancer cells in the culture. In this approach for the first time we investigated the autophagic and apoptotic effects of lentiviruses harboring DSCR1 in breast cancer cells.

Our result has indicated for the first time significant increase in DSCR1 gene expression ($p<0.023$) up to 4.043 fold in breast cancer cells after lentiviral infection respectively. According to results, DSCR1 gene was overexpressed 62% in infected cells. Ectopic expression of DSCR1 induced apoptosis and autophagy in MDA-MB-468 triple negative cancer cell line.

After ectopic DSCR1 expression, the annexin V/PI experiments showed an increase in the number of cells marked positively for Annexin V, revealing cells in early stage apoptosis (21.1%). Simultaneously, there was an increase in double marked cells (Annexin V+/PI+) (45.5%), revealing the presence of cells undergoing late stage apoptosis or necrosis. After infection, expression of pro-apoptotic genes such as Bax was increased to the rate of 3.932 comparing to non-infected cells. In infected cells, decreased expression of anti-apoptotic genes such as Bcl2 to the rate of 0.2 was observed comparing to non-infected cells.

MDA-MB-468 cells infected using LV-DSCR1+ represented intracellular vacuoles consistent with the characteristics of autophagy under invert microscopic observation. Furthermore, infected cells were able to be stained with Acridine Orange, a specific marker for autophagic vacuoles. Confirmatory experiments were performed with Real
time PCR for genes expression analysis of Lc3 and Beclin1 showing that ectopic DSCR1 increased the expression of LC3 and Beclin1. Expression of the autophagic marker genes including Lc3 and Beclin was increased by 3.812 times and 4.52 times in infected MDA-MB-468 compared to the non-infected cells. As statistical analysis with a $p$ value<0.05 indicate the increase or decrease in the expression of these genes in infected cells was significantly valid compared to non-infected cells.

In summary, we have shown for the first time that ectopic DSCR1 expression induces autophagic and apoptotic cell death in MDA-MB-468 defined as a TNBC cell line. We have evidenced that the cytotoxic effects of DSCR1 over expression is caused by autophagy and apoptosis.

5 Conclusion

Based on the results of this experiment, we can conclude that the higher level of apoptosis and autophagy occurred in cancer cells infected by lentiviruses. The phenomenon of apoptosis and autophagy in cancer cells was confirmed by acridine orange and ethidium bromide staining. In summary, evidence that we present in this investigation demonstrated that LV-DSCR1+ can be efficient for apoptosis and autophagy induction and breast cancer treatment.

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References


