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RESEARCH ARTICLE

Dextran-coated iron oxide nanoparticle for delivery of miR-29a to breast cancer cell line

Serap Yalcin

Department of Molecular Biology and Genetics, Kırşehir Ahi Evran University, Kırşehir, Turkey

ABSTRACT

In the last years, miRNAs have been associated with molecular pathways of cancer and other diseases. The change of expression level of miRNA has an inhibitory role in tumorigenesis. Nevertheless, the poor bioavailability of miRNA due to the rapid enzymatic degradation is a critical handicap in cancer therapy. In this study, we designed dextran-coated iron oxide-based nanoparticle for the delivery of miR-29a to breast cancer cells and analyzed its therapeutic efficacy *in vitro*. Results indicated that the presence of dextran-coated magnetic nanoparticles, loaded with miR29a, enhanced the selective delivery of miR-29a. Further, miR-29a complex nanoparticles caused down-regulation of anti-apoptotic genes. These results pave the way for further investigations into the possible use of miR-29a complex magnetic nanoparticles for breast cancer therapy.

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KEYWORDS
MiR-29a; nanoparticles; breast cancer; dextran; apoptosis

1. Introduction

One of the major public health issues is cancer; therefore, overcoming cancer is an immediate priority (Siegel et al. 2012, 2017). The traditional treatment of cancer with minimum side-effects is still challenging. Today, nanotechnology is a strong focus on cancer therapy. Nanomedicine and nano-based delivery systems, to achieve specific cancer targeting and drug delivery, have been developed including polymers, inorganic nanoparticles, etc. (Lasic 1996; Moghimi et al. 2005; Hawkins et al. 2008; Baker 2009; Minelli et al. 2010; Jain and Stylianopoulos 2010; Wang and Thanou 2010; Gaitanis and Staal 2010; Wang et al. 2012; Steichen et al. 2013; Xu et al. 2019). MicroRNAs (miRNAs), regulators of oncogenes and tumor-suppressor genes, are a new way for diagnosis and treatment of cancers (Cantley et al. 1991; Levine et al. 1991).

miRNAs are single-stranded of ~18–25 nucleotides, and non-coding RNA molecule. They play essential roles in various biological processes such as cell proliferation, differentiation, and apoptosis (Farazi et al. 2011; Reid et al. 2011; Giordano and Columbano 2013; Karakatsanis et al. 2013; Sun et al. 2013; Cai et al. 2015; Li et al. 2018).

Mir-29a has been newly discovered tumor suppressor in most cancers. Several studies have shown that miR-29a downregulate oncogenes and/or upregulate tumor suppressors and they induce apoptosis and inhibit proliferation and invasiveness of cancer (Muniyappa et al. 2009; Hesong et al. 2014). However, the therapeutic potential and delivery of miRNAs are limited due to its physicochemical properties, negative charge, hydrophilicity, nuclease degradation, and poor uptake. Considering the great potential benefits of nanoparticles, miRNA based delivery systems have shown great potential for cancer therapy (Srivastava et al. 2011; Kurtanich et al. 2018). For this reason, the application of miRNAs as potential therapeutic agents need convenient delivery systems that effectively protect the miRNAs from nucleases and boost delivery efficiency for the tumor site/cells. In recent years, non-coding RNA complex nanoparticle systems considered more attractive due to their low toxicity and biodegradability.

2. Material and methods

2.1. Materials

Iron (II) chloride tetrahydride (FeCl2.4H2O), iron (III) chloride hexahydrate (FeCl3.6H2O), Dextran, ammonium hydroxide (NH4OH), phosphate buffered saline (PBS), RPMI-1640, fetal bovine serum (FBS), trypsin–EDTA, gentamycin, polyethyleneimine (PEI) were purchased from Sigma-Aldrich Chemie GmbH, (Germany). XTT cell proliferation assay kit (XTT) was supplied by Biological Industries, Israel Beit Haemek Ltd (Israel).

2.2. Preparation and characterization of Dex-MNPs/miR-29a

Dex-MNPs were *in situ* synthesized by the co-precipitation of iron salts in the presence of dextran molecules. The iron salts were dissolved in water and then, the ammonia (32% NH4OH, 25 ml) and dextran (5% (w/v) in water) solutions were added to the mixture very slowly under the nitrogen gas flow by vigorous stirring at 2000 rpm. The resulting solution was stirred for an additional 2 h. The dextran-coated magnetic nanoparticles (Figure 1) were extensively washed with deionized water and separated by magnetic decantation. Synthesized Dex-MNPs were characterized by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), vibrating sample magnetometer (VSM), scanning electron microscopy (SEM) analyses (Unpublished data, Yalcin et al.). Dex-MNPs was dissolved with slight stirring and sonicated in an ice bath. Dex-MNPs solution (25 mg/mL in methanol) was mixed with an aqueous PEI solution (5 mg/mL) and sonicated in an ice bath. Then, the coated MNPs solution was added to the miR-29a solution at the different ratios, mixed gently, and then incubated for 15–20 min at room temperature to form Dex-MNPs/miR-29a. The zeta potential of the Dex-MNPs/miR-29a was determined at 25 °C.

CONTACT Serap Yalcin serapyalcin1982@gmail.com Department of Molecular Biology and Genetics, Kırşehir Ahi Evran University, Kırsehir 40100, Turkey

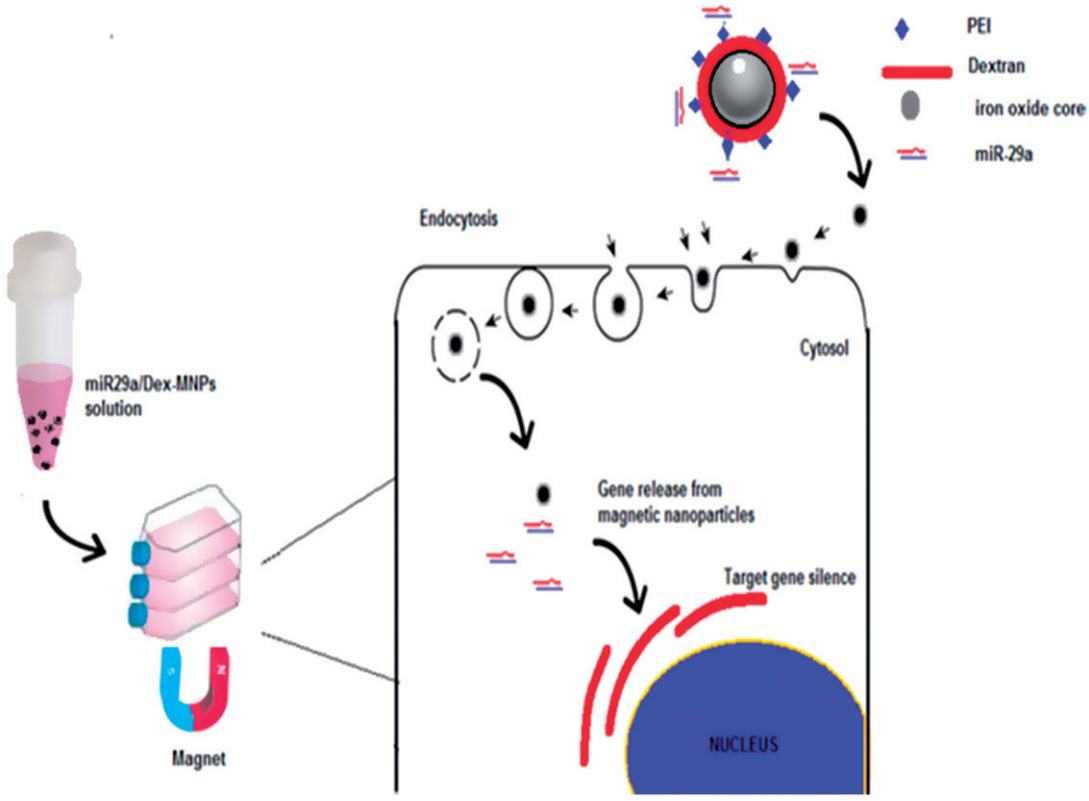

Figure 1. Schematic representation internalization of Dex-MNPs/miR-29a into the cell.

2.3. Determination of property of Dex-MNPs/miR-29a with agarose gel electrophoresis

To detect the binding ability of miR-29a (hsa-miR-29a mimics (sense: 5′-UAGCACCAUCUGAAAUCGGUUA-3′; antisense: 5′-ACCG AUUUCAGAUGGUGCUAUU-3′) to Dex-MNPs the electrophoresis analyses were performed. Dex-MNPs and miR-29a (30 nM) were mixed at Dex-MNPs/miR-29a ratios of 1:1, 2:1, 4:1 and 8:1 (w/w) and incubated at room temperature for 15–20 min. 10 µl of the Dex-MNPs/miR-29a to mixture were loaded onto 1,2% agarose gels with ethidium bromide and run with Tris-acetate running buffer at 150 V for 50 min. The retardation of miR-29a was observed with ethidium bromide staining and photographed under BIORAD gel image analyzer.

2.4. Cellular internalization of miR-29a-Dex-MNPs

The MCF-7 human breast cancer cell line was donated by the Erciyes University, Kayseri, Turkey. The cells were further incubated in RPMI-1640 medium. In order to visualize the cellular internalization of Dex-MNPs/miR-29a on MCF-7 cells were incubated for 6 h. After the cells treated with miR-29a-Dex-MNPs were washed with phosphate buffer saline. Then the images of the cells were taken by inverted microscopy (BAB, Turkey).

2.5. Cytotoxicity miR-29a-Dex-MNPs and Dex-MNPs

Cytotoxicity of miR-29a-Dex-MNPs, Dex-MNPs, Dex-PEI-MNPs, PEI and Dextran polymer on cancer cells (MCF-7) were determined by XTT Cell Proliferation Assay. According to the instructions of the manufacturer, XTT reagent was added after the cells were exposed to different concentrations of miR-29a-Dex-MNPs and Dex-MNPs, for 72 h. The cell viability of control groups was considered %100. Amount of soluble product formazan dye was measured at 496 nm by a microplate reader (BIOTEK) and IC50 values were calculated.

2.6. Expression levels of apoptosis-related genes

Effect of miR-29a-Dex-MNPs on anti-apoptotic (BIRC-5, BRAF, AKT-1 and Bcl-2) genes, and miR-29a expressions were investigated by real time RT–PCR analyses with Roche LightCycler 480 instrument. miR-29a-Dex-MNPs, Dex-MNPs treated and untreated breast cancer cells was performed by using miRNA and RNA isolation reagent (Roche Life Sciences, USA) from (MCF-7). RNA quality and quantity were checked by spectrophotometric analysis. cDNA synthesis was done by using random hexamer primers and qPCR was performed. Each sample amplification was performed in triplicates. A nontemplate control (NTC) was included to identify for any background signal and any possible contamination.

2.7. Statistical analysis

Data analyses were expressed as mean ± standard error of mean (SEM). Statistical analyses were performed with GraphPad Prism 7 software by using ANOVA, with $p < 0.05$ considered to represent statistical significance.

3. Results and discussion

Biocompatible and biodegradable polymers are special in biomedical research due to their low toxicity, low cost, feasibility. Dextran-coated nanoparticles have been widely studied as anticancer agent carriers in biomedical research (Feng 2004; Yang et al. 2012). The presence of hydroxyl groups on the surface of dextran-coated nanoparticles can easily allow attachment with

anti-cancer agents by the linker or direct conjugation. In this study, dextran-coated magnetic nanoparticles were synthesized with a simple, short and efficient method. The synthesized Dex-MNPs were characterized with FTIR, TGA, SEM, zeta potential and VSM analyses. The particle size of Dex-MNPs was ranging between 10 and 15 nm. The morphology of Dex-MNPs was observed fairly smooth and spherical on TEM and SEM images (Unpublished data, Yalcin et al.). The sizes of synthesized miR29a- Dex- PEI-MNPs were found as 25–30 nm in TEM analyses.

The zeta potential values of bare MNPs were calculated as −24.5 mV in pH 7.4. The zeta potentials of Dex-MNPs and miR29a-Dex-PEI-MNPs were observed at +19.8 and +22.4 mV respectively. For MCF-7 cells, the zeta potential was −21.4 mV and thus positively charged NPs exhibited high cellular uptake because of their high affinity to the negatively charged tumor cell membranes. In addition, miR-29a complex Dex-MNPs were investigated on breast cancer cells and analyzed its therapeutic efficacy *in vitro* (Figure 2).

FTIR study was carried out to verify the compatibility between Dextran, Dex-MNPs and MNPs are presented in Figure 3. The FTIR spectrum was measured on the solid as KBr disc/pellets. This spectrum was recorded in the range of 4000–400 cm^{-1} at room temperature with Thermo Scientific Nicolet 6700 FT-IR instrument. The major peaks determined to the Dextran coated MNPs confirm the presence of different groups.

The most commonly used viral vectors for miRNA delivery in target tissue regions. Unfortunately, its degradation by nucleases in biological fluids and poorly transporting to the target site leads to clinical difficulties associated with local administration (Van Rooij and Olson 2012; Li and Rana 2014; Van Rooij and Kauppinen 2014). These limitations have led to the development of alternative approaches to increase the efficacy of in vivo delivery, such as miRNA complex nanoparticles (Chen et al. 2015; Zheng et al. 2018).

In this study, it was clearly observed that miR-29a-Dex-PEI-MNPs are taken up into the cells by endocytosis. The efficacy of Dex-MNPs was demonstrated as a miR-29a delivery vehicle, which is smaller than nuclear pores and enters across the cell and nuclear membrane (Figure 4). According to *in vitro* cytotoxicity analysis, miR-29a-Dex-PEI-MNPs were found as highly apoptotic to cancer cells on cancer cells. The inhibitory concentration of 50% (IC50) cell proliferation values was calculated. The IC50 value of miR-29a-Dex-PEI-MNPs was 5 nM, substantially low. Bare iron oxide (MNPs) and Dex-MNPs had also no effect on cell proliferation up to 1000 μM (Figures 5 and 6).

PEIs, noncovalent complexes with nucleic acids, have positively charge. This system causes protecting small RNAs from degradation, transfection efficacy, low toxicity and lysosomal escape into the cytoplasm (Akinc et al. 2005; Aigner 2007). In this study, we have used the PEI –LMW for nanoparticle synthesis. The performed cytotoxicity assays demonstrated that were nontoxic up to 1000 μM concentrations. miR-29a-Dex-PEI-MNPs inhibited cell proliferation, compared with the free nanoparticles.

Alteration expression levels of miR-29 family have been reported in cancer cells (Kasinski and Slack 2011; Di Leva et al. 2014). The down-regulated expression level of miR-29a reported in breast cancer by many researchers (Wu et al. 2013; Li et al. 2017).

In this study, we evaluated the effect of miR-29a-Dex-PEI-MNPs on the expression levels of BIRC-5, BRAF, AKT-1 and Bcl-2 in MCF-7 cell line. According to our results, BRAF, AKT-1, and BIRC-5 genes expression were up-regulated on untreated cancer cells.

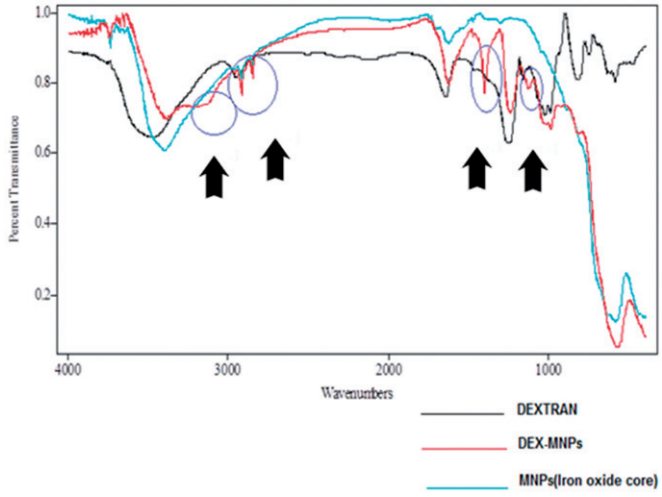

Figure 3. Comparison of FT–IR results of Dextran, Dex-MNPs and MNPs.

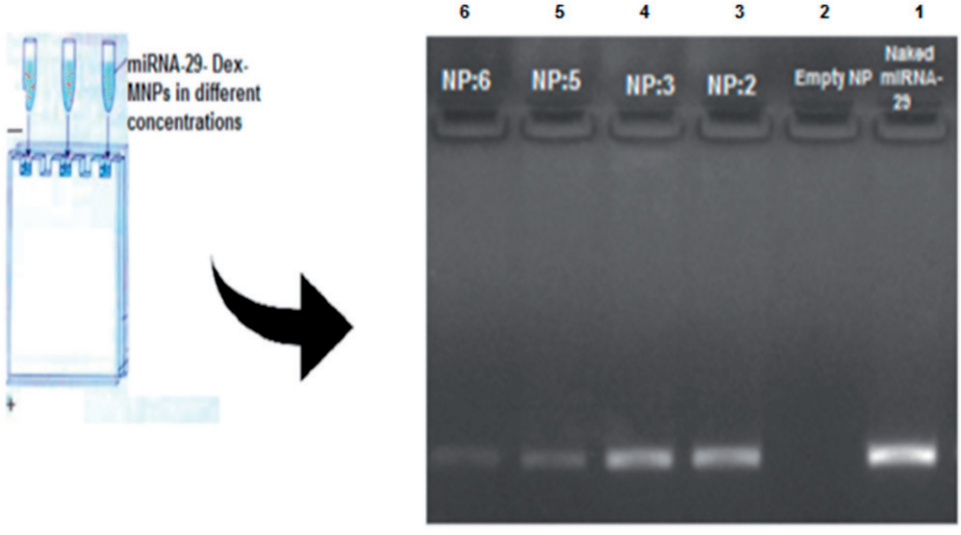

Figure 2. Agarose gel (1.2%) of miR29a-Dex-PEI-MNPs (1:1, 2:1, 4:1 and 8:1 w/w ratios) complexes (lane 3–6). miRNA alone (lane 1) and free Dex-MNPs (line 2) were run as controls.

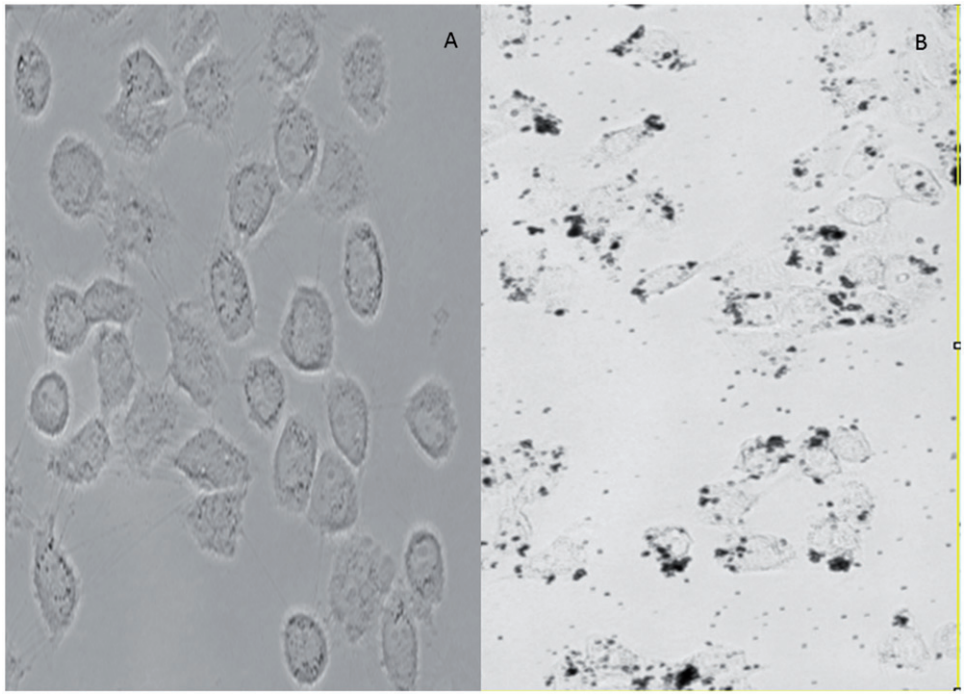

Figure 4. (A) Control (B) Cellular internalization of miR-29a-Dex-PEI-MNPs on MCF-7.

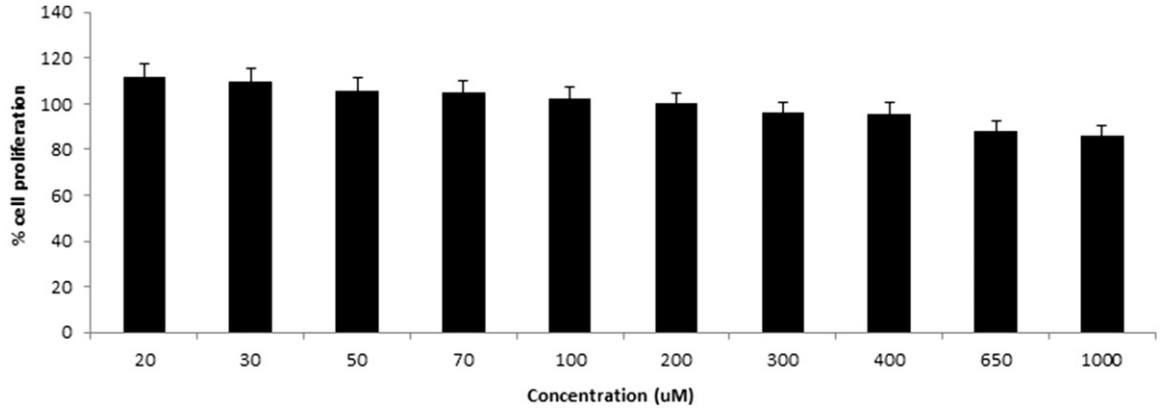

Figure 5. XTT assay graph of Dex-PEI-MNPs on MCF-7 cell line.

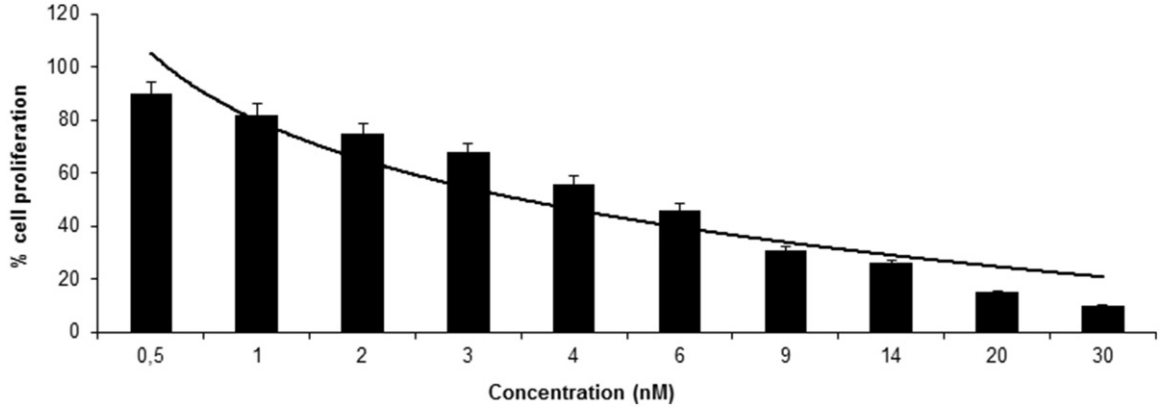

Figure 6. XTT graph of mir-29a-Dex-PEI-MNPs on MCF-7.

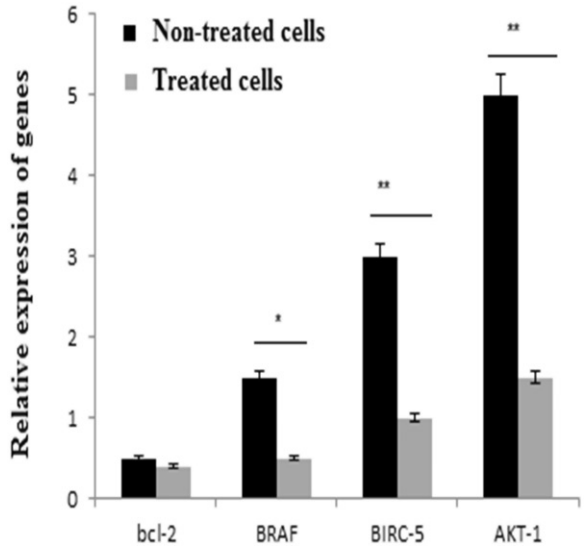

Figure 7. The expression levels of Bcl-2, BRAF, BIRC-5, AKT-1 genes in MCF-7 cells treated with miR-29a-Dex-PEI-MNPs.

We observed that the anti-apoptotic AKT-1 and BIRC-5 genes were down-regulated in response to miR-29a-Dex-MNPs in cancer cells. So, BRAF, AKT-1, and BIRC-5 down-regulation could contribute to apoptosis of miR-29a-Dex-MNPs treated breast cancer cells (Figure 7). Whereas, expressions levels of Bcl-2 gene did not change significantly between the treated and untreated cell line.

Conclusion

miRNAs have been investigated currently as therapeutic targets for cancer by scientists (Kong et al. 2012; Hayes et al. 2014). The human miR-29 family of microRNAs has three mature members, miR-29a, miR-29b, and miR-29c. Many studies have reported that miR-29b and miR29c loaded/complex with nanoparticle on the various cancer type (Huang et al. 2013; Perepelyuk et al. 2018) but up until now the studies have not been conducted showing the effect of miR-29a loaded/complex magnetic nanoparticles on this day. MicroRNAs have a short half-life and poor stability. To overcome this issue, we have developed miR-29a with Dextran-coated magnetic nanoparticles systems for cancer therapy. According to the results of the study, we showed that Dex-MNPs formulation can be a safe alternative for drug delivery and the usage of magnetic nanocarriers may protect miRNAs from cellular nucleases and effectively carry miRNA to cancer cells *in vitro*/in vivo. These results suggest that miR-29a can serve as a potential therapeutic target for breast cancer. Further evaluation studies will be required to investigate the bioavailability of miR-29a loaded nanoparticles in vivo model.

Disclosure statement

No potential conflict of interest was reported by the author.

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