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Influence of siRNA Loaded Nanoparticles on the Metastasis and Growth Rate of Cancer Cell Lines

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ABSTRACT

Objectives: The aim of this study was to investigate the ability of siRNA-loaded nanoparticles to control the capability of cisplatin-resistant cell lines to decrease cancer metastasis and cell proliferation rate. **Methods:** The effect of siRNA-loaded NPs on the migratory (metastasis) and proliferation (cell growth rate) activity of cisplatin-resistant cell lines compared to untreated cells was investigated using the wound healing assay and cell growth rate assay, respectively. **Results:** The results showed that cells were treated with siRNA-loaded nanoparticles after 24 h from starting the scratch, succeeded to cover of 59 ± 12.9, and 30 ± 6.1% only from the wound for cell line Cis 1R and Cis 12R, respectively. On the other hand of the untreated cells that were treated with positive control nanoparticles showed complete healing for the wound within the same period of time. Results of cell proliferation of both cell lines exhibited significantly ($P \leq 0.001$) lower growth rate after treatment with loaded nanoparticles compared to positive control nanoparticles and untreated cells. **Conclusion:** The obtained results indicated that siRNA-loaded nanoparticles succeeded to not only reduce metastasis but also decrease cell proliferation rate of cisplatin-resistant cell lines.

Keywords: *Cell growth, Migration, Proliferation, Scratch assay, siRNA.*

INTRODUCTION

Cisplatin is a common chemotherapeutic agent that has been used for treatment of different types of cancer such as tumor bladder, head and neck, lung, and ovarian cancers[1]. DNA represents the primary target for interaction of cisplatin with the cell [2]. The mode of action depends on different strategies and pathways such as its ability to cross link with the purine base on the DNA backbone, its interfering with mechanism of DNA repairing, and ability to cause DNA damage which induces cancer cell apoptosis[1].

The main complications of the cancer chemotherapy are the resistance, metastasis, and tumor relapse. Resistance to cisplatin and the distant metastasis are governed by a subpopulation of cancer cells called cancer stem cells (CSCs) which was discovered and fully identified through different studies in different types of carcinoma. The main characteristics of CSCs include being less differentiated cells with high proliferation rate, metastasis to the distant organs and having self-renewal ability as well as expressing resistance to chemo and radiotherapy[3,4,5,6].

CSCs can be identified by detection and measurement of stem markers which may be found at the surface of the cells (surface stem markers) like CD133 and CD44+ proteins, or may be at nucleus side like Bmi-1 protein. These stem markers play an important role and

control the main features of the CSCs[5]. Bmi-1 or B-cell specific Moloney murine leukemia virus insertion site-1, is a stem cell gene that was identified in 1990 as a crucial component of the Moloney murine leukemia virus[7]. Different studies approved the relationship between over expression of Bmi-1 protein and different types of cancers such as leukemia, ovarian cancer, and head and neck cancer[8,9,10].

Bmi-1 is essential for epithelial-mesenchymal transition (EMT) during the development of cancer cells in large number of cases. Induction of EMT is responsible for dissemination of cancer cells from primary tumor to distant organs, activation of self-renewal, and resistance of cancer cells for chemotherapy[11,12]. Also, the role of Bmi-1 in the proliferation of stem cells that are involved in tumorigenesis was proven through different experimental studies[13]. Therefore, the main thoughts were about the knockdown of Bmi-1 protein expression through targeting the translation of mRNA with siRNA and the expected results to control the growth rate and migration capability of CSCs.

Different categories of carriers were tested to condense siRNA by electrostatic interaction forming ionic complexes that are internalized to the tumor cells by endocytosis like cationic lipids, polymers, and peptides. These carriers had faced many limitations such as escaping the endosomal/lysosomal trap or requiring unreasonably high amount of carrier to deliver siRNA, which results in carrier cytotoxicity, or opsonization by reticuloendothelial organs[14,15]. Nanoparticles (NPs) are considered as a reasonable delivery system that could protect siRNA from degradation by nucleases enzymes upon systemic injection and able to release the loaded siRNA to the cytoplasm of the required cells[16].

The main aim of the work was to investigate the ability of siRNA-loaded nanoparticles (siRNA-loaded NPs) to affect the main characters of CSCs such as metastasis via migration to the distant organs and self-renewal through high growth rate.

MATERIALS AND METHODS

Materials and Cell lines

Signal silence Bmi-1 siRNA was purchased from Cell Signal Technology (CST), Danvers, Massachusetts, USA. Silencer negative control siRNA was purchased from Ambion, California, USA. Bmi-1 siRNA and negative control siRNA-loaded NPs were obtained by complexation with modified β-cyclodextrin polymer according to established protocol[17]. Dulbecco's modified Eagle's medium DMEM (high glucose, pyruvate), OPTI-MEM reduced serum medium, fetal bovine serum (FBS), trypan blue 0.4%, and phosphate buffered saline (PBS) were purchased from Gibco by life technologies, New York, USA. Cisplatin-resistant cell lines, UM-SCC-22B Cis 1R (Cis 1R) and UM-SCC-22B Cis 12R (Cis 12R), were kindly provided as a gift from Dr. Jacques Nor, School of Dentist, University of Michigan, USA.

Methods

The effect of siRNA-loaded NPs on the migratory (metastasis) and proliferation (cell growth rate) activity of cisplatin-resistant cell lines compared to untreated cells was investigated using the wound healing assay and cell growth rate assay, respectively.

Wound healing assay

The experiment was carried out according to established protocol with some modifications[16,18,19]. Cisplatin-resistant cell lines were plated in 6-well plates at a seeding density of 300,000 cells per well and allowed to adhere for 18 h overnight. The adherent cells were incubated with siRNA-loaded NPs and nanoparticles loaded with negative sequence of siRNA (positive control NPs) for 6 h in serum-free OPTI-MEM followed by addition of 1 ml of fresh culture medium to each well. After 12 h of incubation, the culture medium was aspirated and replaced with serum-free OPTI-MEM containing the same treatments for a second incubation cycle. After a total of 24 h, cells were washed with phosphate buffer saline (PBS) pH 7.4, trypsinized using 0.25% Trypsin/EDTA, and centrifuged to collect the cell pellets before suspending them in standard culture medium at a density of 50,000 cells per ml. The collected cells were plated in 24-well plates at a seeding density of 50,000 cells/well and allowed to grow under normal culture conditions to 95% confluence. The cell monolayer was scratched with a sterile 200 µl pipet tip to create a "wound" across the center of each well. Each well was washed with PBS to remove the detached cells. The created wound was imaged at a 4× magnification using a Nikon Eclipse Ti inverted microscope **(**Minato-ku, Tokyo, Japan) equipped with a camera to capture phase contrast images of the wound at different time points 0, 12, and 24 h. The acquired images were processed using ImageJ software (NIH, Bethesda, MD) to calculate the area that was occupied from the wound at each time point. The wound area after 24 h was normalized to the initial area (0 h) to quantify the change in cell migration in response to different treatment. Results represent the average occupied wound area for six wells ± SEM.

Cell growth rate assay

The main principle of cell growth rate assay experiment is similar to the cell viability assay, which relies on the change of the resazurin dye from blue color to the fluorescent pink color as response to the metabolic rate of the cells[20]. Cisplatin-resistant cell lines were treated for two incubation cycles as previously described.

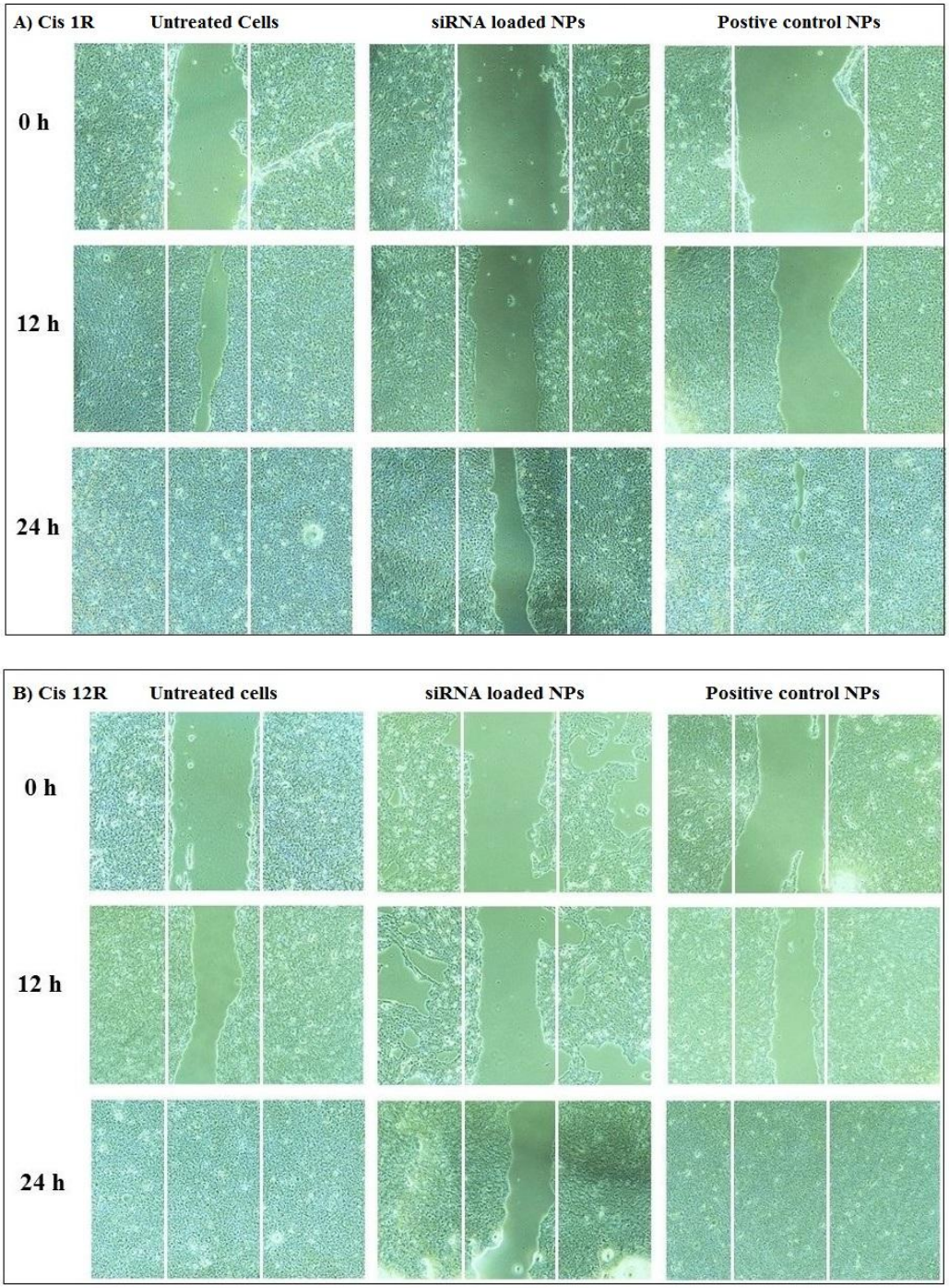

Figure 1: Representative images (4× magnification) showing the effect of siRNA-loaded NPs and positive control NPs on the migration of (A) UM-SCC-22B Cis 1R cells and (B) UM-SCC-22B Cis 12R cells.

The collected cells were plated in 96 well plates with cell density 1500 cell/well and were incubated at 37ºC and 5% CO_2. After 18 h from seeding, the first plate was processed by aspirating medium and addition of 200 µl of resazurin solution (20µg/ml) followed by incubation for 4 h. The fluorescence of the first plate was obtained using a microplate Fluoroskan reader (ASCENT, Finland) and used as a base line for further cells growth in the next days. The daily growth rate of cells was calculated as fluorescence ratio compared to fluorescence of basal line. Results were represented as average of three independent experiments ± SEM.

Statistical analysis

In order to compare the results, Student's t test (SPSS program; version 12.0, SPSS Inc., Chicago, USA) was used. Data were reported as means ± SEM. A statistically significant difference was considered at *P* value < 0.05.

RESULTS AND DISCUSSION

Wound healing assay

The wound-healing assay was carried out to investigate the change in cell migration in response to different treatments following established protocols. The obtained images for the wells show that Cis 1R **(Figure 1A)** and Cis 12R cell lines **(Figure 1B)** treated with positive control NPs retained their migration ability as observed by covering of the scratch after 12 h and 24 h. These images are closely similar to that of the untreated cells (control group). On the other hand, the images of cells that were treated with SiRNA-loaded NPs showed partial covering of the scratched area at the same time points. The calculated % of occupied areas (mean ± SEM) in scratches of Cis 1R cell line after 12 h were 29 ± 3.8, 50 ± 13.4, and 67 ± 3.4%. However, after 24 h, the % occupied areas significantly increased to 59 ± 12.9, 96 ± 7.2 and 100 ± 0.5 % for Bmi-1 siRNA-loaded NPs, positive control NPs and for negative control, respectively. These results revealed that siRNA-loaded NPs were significantly ($P \leq 0.05$) able to slow down cell proliferation and mobility of Cis 1R cell line compared to both positive control NPs and untreated cells (Figure 2). The obtained results also indicated that the positive control NPs were not able to change the % occupied area compared to the untreated cells, indicating that the nanoparticle as a carrier for siRNA has no cytotoxicity and the change in cell migration was due to the effect of siRNA only. Similar results have been observed in case of Cis 12 R cell line

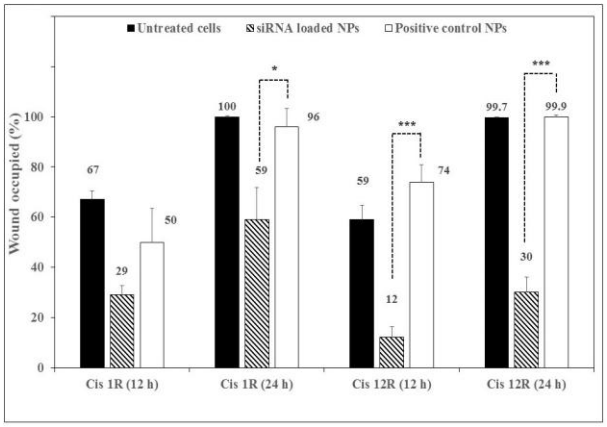

Figure 2: Wound occupied (%) of two Cis 1R and Cis 12R cell lines after 12 and 24 h of treatment with siRNA-loaded NPs, positive control NPs compared to untreated cells normalized to the initial wound (t = 0 h).

Cell growth rate

The experiment was carried out to evaluate the effect of siRNA-loaded NPs on the cell growth rate of cisplatin-resistant cell lines. The daily growth rate of cells was expressed as fluorescence ratio compared to fluorescence of fist day growth as a basal line. The daily growth rate of Cis 1R cell line treated with siRNA-loaded NPs, positive control NPs, compared to the untreated cells is represented in Fig 3. The growth rate for the siRNA-loaded NPs treated cells was significantly reduced ($P \leq 0.001$) compared to either untreated cells or in case of positive loaded NPs treated cells. Similar results (Fig 4) have been obtained with Cis 12R cell line. The obtained results illustrated that the positive control NPs was not able to affect growth rate of both cisplatin-resistant cell lines compared to the untreated cells, indicating that the nanoparticle as a carrier for siRNA has no cytotoxicity. Moreover, the results confirmed that the reduction in the growth rate was due to the effect of siRNA not the carrier.

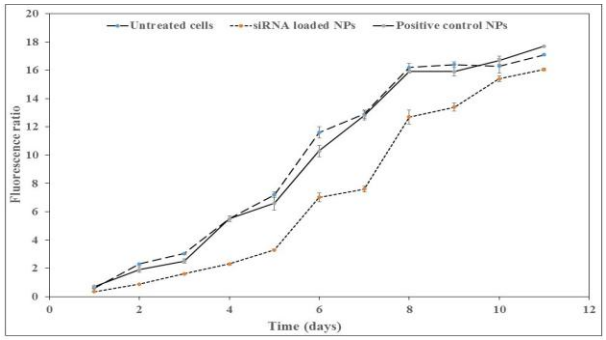

Figure 3: Growth rate of Cis 1R cell line for untreated, positive control NPs treated cells and siRNA-loaded NPs treated cells.

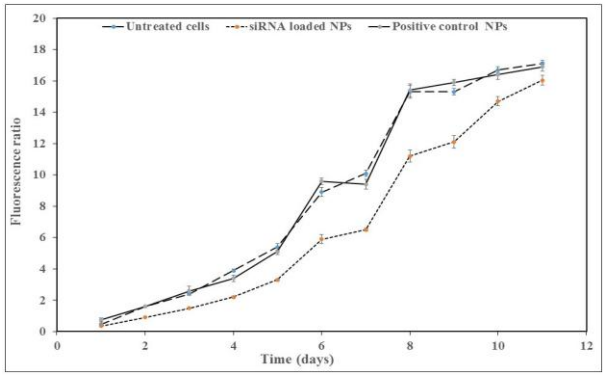

Figure 4: Growth rate of Cis 12R cell line for untreated, positive control NPs treated cells and siRNA-loaded NPs treated cells.

CONCLUSION

The obtained results indicated that siRNA-loaded nanoparticles succeeded to reduce metastasis and cell proliferation rate of cisplatin-resistant cell lines.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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