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Overcoming Multidrug Resistance of Triple Negative Breast Cancer Cell Lines using miRNA 374c-5p and its Inhibitor-Chitosan Nanoparticle Conjugates

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KEYWORDS

Drug resistance, breast cancer, chitosan, nanoparticles, gene delivery

SHORT SUMMARY

Non-coding RNA (miRNA), when combined with chitosan nanoparticles CNP, has been proven to be an efficient gene delivery approach to overcome drug resistance for triple negative breast cancer TNBC cases.

EXTENDED ABSTRACT

Background

Breast cancer is considered the 2nd highest cancer in incidence, accounting for 38.8% of all the female cases of cancers recorded in Egypt. Amongst the molecular subtypes, triple negative breast cancer (TNBC) is the most aggressive one, constituting 15-20% of breast cancers and associated with earlier age of onset, aggressive clinical course, and multidrug resistance.

The emergence of non-coding RNAs (e.g., miRNAs) as crucial regulators of gene expression has raised exciting possibilities towards them being explored as alternative. Recently, miR-374c-5p is gaining interest especially its effect on cell proliferation, migration, and epithelial-mesenchymal transition with concomitant induction of apoptosis. However, the role of miRNA-374c-5p in breast cancer deserves careful verification, which may be a novel and vital marker for cancer diagnosis and various treatment strategies.

The delivery of miRNAs into tumor location has been limited by many barriers: such as their possible degradation by nucleases, low cellular uptake, poor endosomal release, and elimination by phagocytic immune cells. Non-viral delivery systems like chitosan nanoparticles (CNPs) have been developed to overcome these barriers. The development of targeted cancer therapeutics is being reinvented by safe multifunctional nanocarriers based upon miRNA delivery. CNPs appear to be effective delivery vehicles to achieve these goals.

Aim of the work

The aim of this study was to investigate the potential utility of miR-374c-5p CNPs conjugates in bypassing multidrug resistance in triple negative breast cancer cell line (MDA-MB-231).

Materials and methods

The study was a multi-faceted research work consisting of an *in vitro* study on TNBC cell lines; MDA-MB-231 testing the efficiency of miRNA CNPs conjugates in sensitizing the cell lines to chemotherapeutic drugs (paclitaxel and doxorubicin; Pac and DOX). CNPs were prepared and characterized for their zeta size and zeta potential and imaged by the transmission electron microscope whereas cellular uptake was assessed using the confocal microscopy. After delivery of miRNA, cell viability was evaluated through cytotoxicity, and cells were characterized by Ki67 immunohistochemical staining, and wound healing assays, along with cell cycle analysis.

The second arm of the work was a retrospective study focused on the expression of miR-374c-5p and its downstream target genes (Cyclin D1, MID1, and Caspase 3) from archived paraffin blocks of triple negative breast cancer patients who have received neoadjuvant chemotherapy, and correlation of the expression with. Expression levels were correlated to the clinicopathological parameters of the patients.

Results

Dynamic light scattering techniques confirmed the successful synthesis and loading of CNPs with miR-374c-5p. Upon transfection, CNPs showed similar delivery efficiency to standard Hiperfect transfection agent. It was of interest that the basal expression of miR 374C-5p target genes Cyclin D1 (CCND1) and MID1were higher in MDA-MB-231 cells than MCF7 cells. Transfection using miR374C-5p led to successful downregulation of MID1 and CCND1 target genes in MDA-MB-231 cell.

miR374C-5p showed a cytoprotective effect on MDA-MB-231 cells when delivered by either HiPerfect or CNPs, however, miR374C-5p transfection did not augment the cytotoxic effects following Paclitaxel and Doxorubicin treatment, respectively. Nevertheless, the anti-miRNA altered the cytotoxicity following Paclitaxel and Doxorubicin treatment in a dose-dependent manner.

Proliferation assays revealed an increased cellular proliferation rate in cells transfected with miR374C-5p following Paclitaxel and Doxorubicin treatment (48.87±10.97), compared to controls (22.47±0.0). On the other hand, assessment of apoptosis using Annexin V showed no significant effect on apoptosis in cell lines transfected with miR374C-5p following Paclitaxel and Doxorubicin treatments. Whereas cell apoptosis and migration were affected following transfection with anti-miRNA after Paclitaxel and Doxorubicin treatments.

Triple negative breast cancer patient tissues showing no or partial response to neoadjuvant chemotherapy showed significantly higher expression of MID1 gene, whereas patients achieving a complete pathological response showed reduced expression.

Conclusions

The CNPs have proven to be an efficient gene delivery platform compared to commonly utilized, commercially available gene transfection agents, such as Hiperfect. CNPs alone have shown to exert a cytotoxic effect on both MCF7 and MDA-MB-231 cells. *CCND1* and *MID1* genes regulated by miRNA 374-5P are upregulated in TNBC patients with partial or no response to neoadjuvant therapy, indicating its poor prognostic potential. Anti-miR374C-5p augmented the cytotoxic effects of Pac and DOX and holds much promise as an adjuvant treatment modality to overcome multidrug resistance in TNBC.