

## LETTER TO THE EDITOR

# Role of *eIF4E* on epithelial-mesenchymal transition, invasion, and chemoresistance of prostate cancer cells

**Dear Editor,**

Prostate cancer (PCa) is the most common cancer and second leading cause of cancer death for men in the United States [1]. PCa with similar Gleason score has been reported to show substantial interpatient heterogeneity and differential prostate cancer-specific mortality rate [2]. Such heterogeneity in PCa often results in different therapeutic responses among patients, including therapy resistance, therapeutic failure, relapse, and metastasis [3]. Numerous oncogenes, such as eukaryotic translation initiation factor 4E (*eIF4E*), have been reported to be involved in epithelial-mesenchymal transition (EMT) and/or drug resistance in PCa [4]. We previously demonstrated that *eIF4E* overexpression was involved in chemoresistance of triple-negative breast cancer and silencing *eIF4E* significantly inhibited cancer cell proliferation and sensitized cancer cells to chemotherapy in a patient-derived xenograft mouse model [5]. In addition, *eIF4E* phosphorylation is known to stimulate the translation of matrix metalloproteinase 3 (MMP3) and Snail mRNAs to promote EMT in PCa [6]. Furthermore, the complexity and dynamic nature of EMT contributes to the heterogeneity of aggressive cancer cells [7]. The precise role of *eIF4E* in EMT, invasion, and chemoresistance in PCa is still to be established with consideration of different subpopulations in order to develop precision medicine for PCa. In this work, we aimed to explore the role of *eIF4E* in EMT, invasion, and chemoresistance in PCa for establishing a promising new therapeutic strategy by regulating *eIF4E* expression using (1-aminoethyl)iminobis[N-oleicylcysteinyl-1-aminoethyl] propionamide] (ECO)/small interfering RNA (siRNA) nanoparticles previously developed in our lab [8-10] for PCa therapy in the context of tumor heterogeneity.

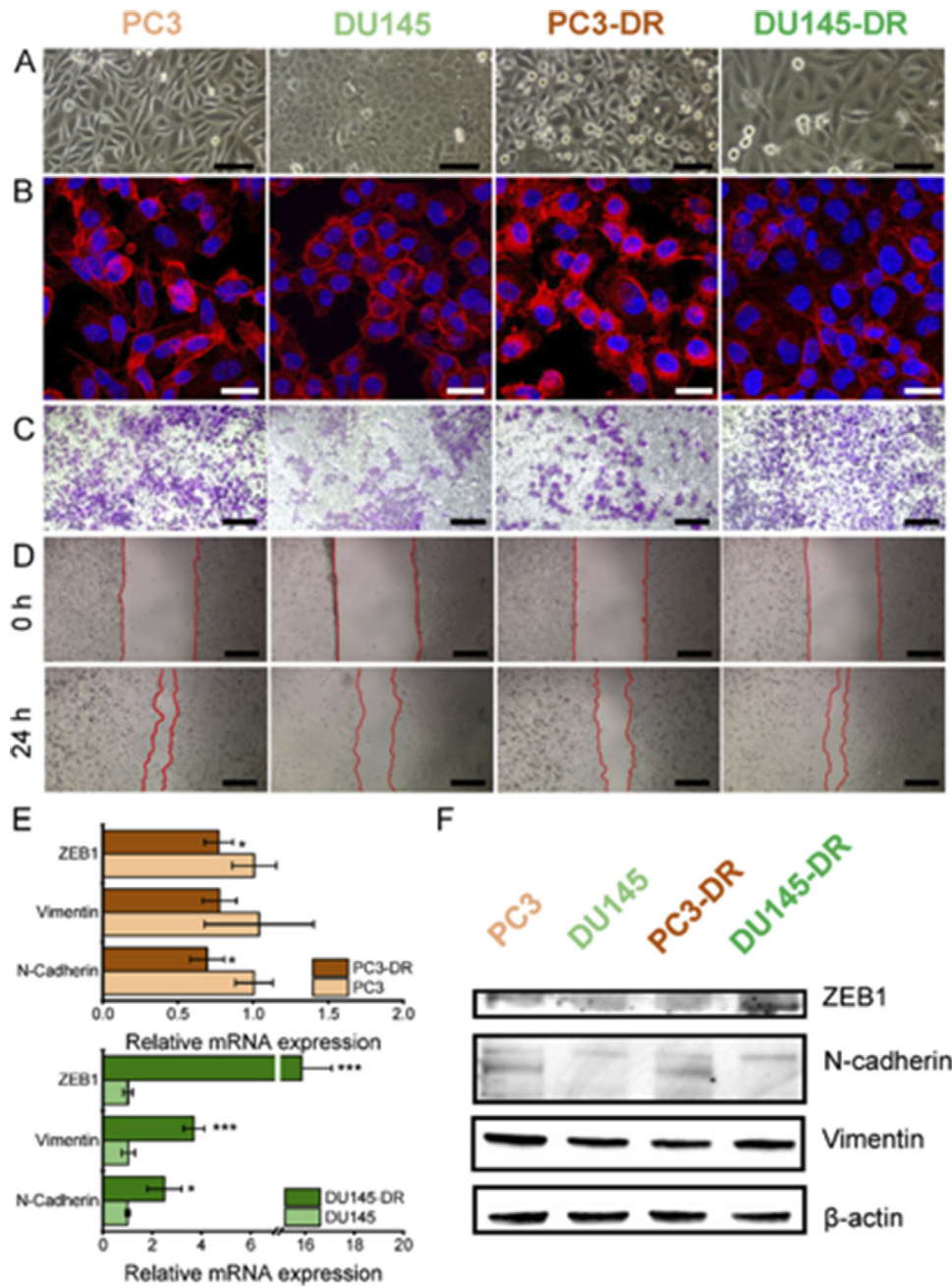
Two PCa cell lines (PC3 and DU145) and their corresponding paclitaxel (PTX)-resistant cell lines (PC3-DR and DU145-DR) were investigated to assess the role of *eIF4E*

in EMT, invasion, and chemoresistance of PCa cells (more details in Supplementary information). The average  $IC_{50}$  of PTX for PC3-DR cells (577.88 nmol/L) was about 69 times higher than that for PC3 cells (8.37 nmol/L), and the average  $IC_{50}$  of PTX for DU145-DR cells (379.15 nmol/L) was about 85 times higher than that for DU145 cells (4.44 nmol/L), indicating that both the drug-resistant cell lines acquired significant resistance to PTX treatment. Interestingly, PC3-DR and DU145-DR cells displayed different morphology and invasiveness from their corresponding parental cell lines (Figure 1A-D). PC3 cells were inherently invasive and displayed spindle-like mesenchymal morphology, while PC3-DR cells exhibited a squamous or epithelial morphology (Figure 1A and B). In addition, wound healing and transwell invasion assays revealed that PC3-DR cells were less migratory and invasive than PC3 cells (Figure 1C and D, Supplementary Fig. S1). On the contrary, DU145 cells displayed the epithelial hallmark of densely packed squamous morphology, whereas DU145-DR cells exhibited an elongated and spindle-like morphology, which is characteristic of mesenchymal cells (Figure 1A and B). DU145-DR cells were more invasive than DU145 cells, as evidenced by an increase in migrated cells in transwell migration assay and narrower gap in wound healing assay at 24 h (Figure 1C and D, Supplementary Fig. S1). Moreover, significant down-regulation of mesenchymal markers, such as N-cadherin and ZEB-1, was observed in PC3-DR cells as compared to PC3 cells. On the other hand, significant up-regulation of N-cadherin, vimentin, and ZEB-1 was observed in DU145-DR cells as compared to DU145 cells (Figure 1E and F). Taken together, these results demonstrated that although both PC3-DR and DU145-DR cells developed significant chemoresistance to PTX, only DU145-DR cells acquired features of EMT, characterizing by the transition to mesenchymal phenotype and increased invasion in response to PTX resistance. For the inherently mesenchymal PC3 cell

**Abbreviations:** ECO, (1-aminoethyl)iminobis[N-oleicylcysteinyl-1-aminoethyl] propionamide]; *eIF4E*, eukaryotic translation initiation factor 4E; EMT, epithelial-mesenchymal transition; MET, mesenchymal-epithelial transition; MMP3, matrix metalloproteinase 3; PCa, prostate cancer; PTX, paclitaxel; siRNA, small interfering RNA.

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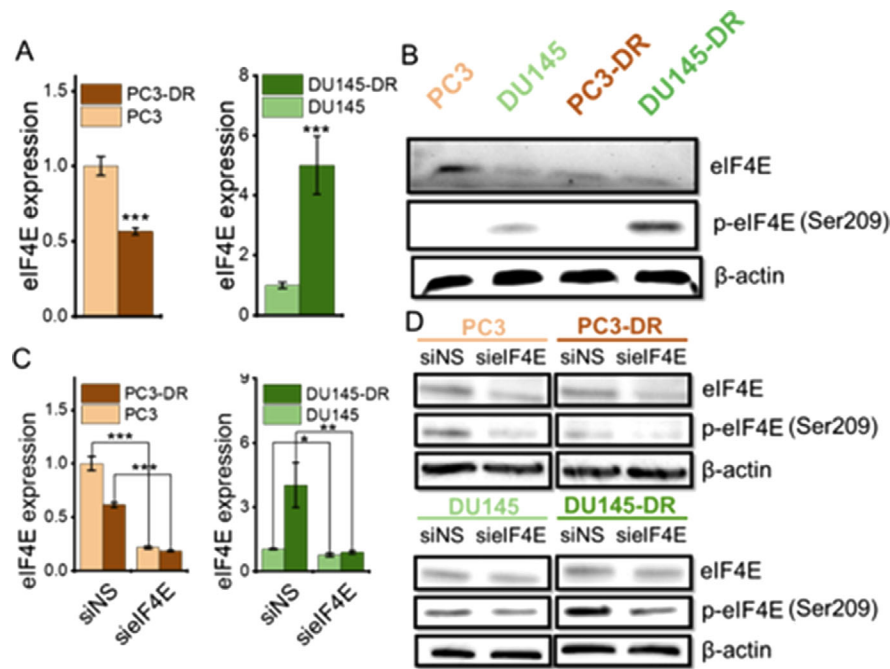


**FIGURE 1** Cell morphology, migration and invasion ability, and EMT features of parental prostate cancer cell lines (PC3 and DU145) and their corresponding paclitaxel-resistant cell lines (PC3-DR and DU145-DR). A. Representative images of cell morphology under microscope. Scale bar: 100  $\mu$ m. B. Representative images of cytoskeleton immunofluorescence staining using anti-F-actin (red) antibody. Nuclei were labeled with DAPI (blue). Scale bar: 100  $\mu$ m. C. Cell invasion ability assessed by transwell invasion assays. Scale bar: 200  $\mu$ m. D. Cell migration potential assessed by wound healing assays. Scale bar: 500  $\mu$ m. E-F. Expression of EMT markers at mRNA (E) and protein (F) levels determined by semiquantitative real-time PCR and Western blotting, respectively.  $\beta$ -actin was used as loading control. The data are represented as mean  $\pm$  SD from 4 independent experiments. \* $P$  < 0.05, \*\*\* $P$  < 0.001, vs parental cells. Abbreviations: EMT, epithelial-mesenchymal transition

line, its corresponding PTX-resistant cell line PC3-DR underwent mesenchymal-epithelial transition (MET) and acquired epithelial morphology with decreased migration and invasion.

Similar to mesenchymal markers, significant down-regulation of eIF4E expression was observed in PC3-DR cells as compared to PC3 cells. Conversely, DU145-DR cells

showed up-regulated eIF4E expression and phosphorylation as compared to DU145 cells (Figure 2A and B). The expression of eIF4E followed the same trend with the expression of mesenchymal markers in all four cell lines, regardless of drug resistance. Considering the results of morphology, EMT, and invasiveness of all four cell lines, it was found that eIF4E



**FIGURE 2** eIF4E expression in prostate cancer cells. A-B. Expression of *eIF4E* in parental prostate cancer cell lines (PC3 and DU145) and their corresponding paclitaxel-resistant cell lines (PC3-DR and DU145-DR) at mRNA (A) and protein (B) levels determined by semi-quantitative real-time PCR and western blotting, respectively. C-D. The *eIF4E* expression at mRNA (C) and protein (D) levels in parental prostate cancer cell lines (PC3 and DU145) and their corresponding paclitaxel-resistant cell lines (PC3-DR and DU145-DR) after treatment with ECO/siNS and ECO/*siEIF4E* nanoparticles determined by semi-quantitative real-time PCR and western blotting, respectively.  $\beta$ -actin was used as loading control. The data are represented as mean  $\pm$  SD from 4 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , vs parental cells. Abbreviations: ECO, (1-aminoethyl) iminobis [N-(oleicysteinyl-1-amino-ethyl) propionamide]

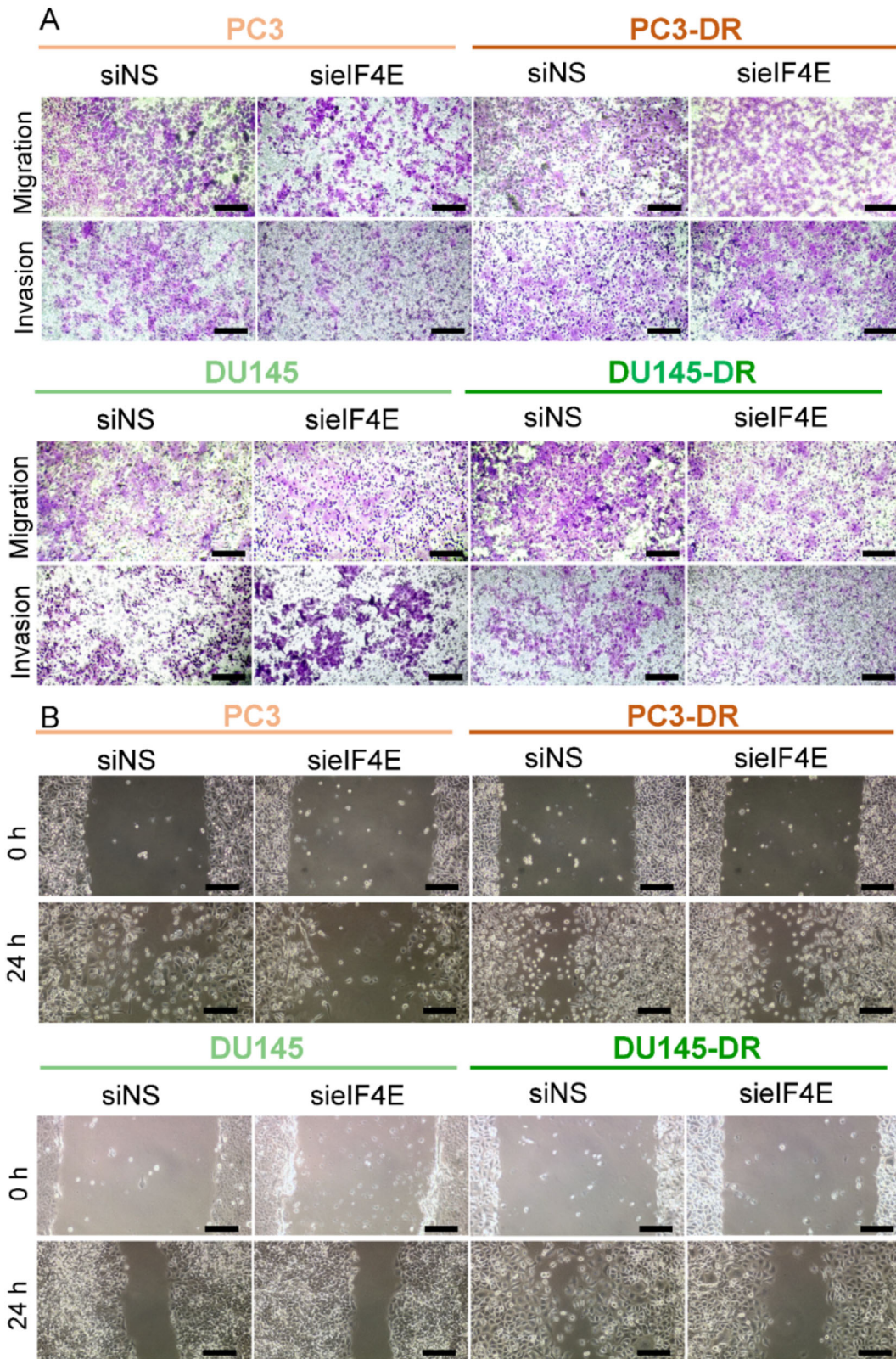
expression was associated with EMT of PCa cells, irrespective of whether they were PTX-resistant or not, indicating a possible role of *eIF4E* in EMT and invasion and a limited role in the development of PTX resistance of PCa cells. Regarding the tumor heterogeneity of PCa, different subtypes of PCa may develop PTX resistance through different routes.

ECO/siRNA nanoparticles have been shown to mediate effective RNAi of various oncogenic targets *in vitro* and *in vivo* [8-10]. To further confirm the role of *eIF4E* in PCa cells, the effects of down-regulation of *eIF4E* were assessed using RNAi based on ECO/siRNA nanoparticles. A multifunctional pH-sensitive amino lipid ECO (Supplementary Fig. S2A) was employed as a carrier to formulate stable nanoparticles with *siEIF4E* via self-assembly for silencing *eIF4E* in PCa cells. Both ECO/*siEIF4E* and ECO/siNS (control) nanoparticles displayed uniform size distribution and positive zeta potential (Supplementary Fig. S2B and C). The gel retardation assay demonstrated efficient encapsulation of siRNA in the nanoparticles, with negligible free siRNA bands (Supplementary Fig. S2D). Compared to the negative control ECO/siNS nanoparticles, ECO/*siEIF4E* nanoparticles resulted in significant *eIF4E* down-regulation in all four PCa cell lines, both at mRNA and protein levels, with more significant silencing in the eIF4E-rich PC3 and DU145-DR cells (Figure 2C and D).

ECO/*siEIF4E* treatment significantly enhanced the response of PC3 and DU145-DR cells to PTX at low concentrations when compared with the ECO/siNS treatment. As for DU145 and PC3-DR cells, which displayed epithelial features and low expression of *eIF4E*, ECO/*siEIF4E* treatment did not show a significant change in response to PTX treatment, compared with ECO/siNS treatment (Supplementary Fig. S3A). The average  $IC_{50}$  of PTX for ECO/*siEIF4E*-treated DU145-DR cells (236 nmol/L) was about 35% less than that for ECO/siNS-treated DU145-DR cells (365 nmol/L), suggesting that silencing *eIF4E* can resensitize PTX-resistant DU145-DR cells. However, treatment with ECO/*siEIF4E* did not have the same resensitizing effect on PC3-DR cells, possibly due to their low *eIF4E* expression and epithelial nature. Silencing *eIF4E* with ECO/*siEIF4E* nanoparticles also resulted in changes in molecular signatures of PC3 and DU145-DR cells. Reduced expression of the mesenchymal markers, ZEB-1, N-cadherin, and vimentin, was observed in the western blots of ECO/*siEIF4E*-treated cells. In contrast, there was no change in these markers in DU145 and PC3-DR cells after treatment with ECO/*siEIF4E* (Supplementary Fig. S3B).

ECO/*siEIF4E*-treated PC3 and DU145-DR cells also showed a significant reduction in their migration and invasion abilities, evidenced by the significantly reduced





**FIGURE 3** Cell migration and invasion ability of normal prostate cancer cell lines (PC3 and DU145) and their corresponding paclitaxel (PTX)-resistant cell lines (PC3-DR and DU145-DR) after treatment with ECO/siNS and ECO/sielF4E nanoparticles. A) Cell migration and invasion ability assessed by transwell study. Scale bar: 200  $\mu$ m. B) Cell migration potential assessed by standard scratch-wound assays. Scale bar: 200  $\mu$ m.

number of migrated cells stained in purple compared with those treated with ECO/siNS (Fig. 3A, Supplementary Fig. S4). The ECO/si*IF4E*-treated PC3 and DU145-DR cells also migrated slower than those treated with ECO/siNS in wound healing assay (Fig. 3B, Supplementary Fig. S5). For DU145 and PC3-DR cells, ECO/si*IF4E* treatment did not result in significant changes in migration or invasion, compared with ECO/siNS treatment (Fig. 3A, Supplementary Fig. S4). Moreover, no significant differences were observed in the wound closure between ECO/siNS and ECO/si*IF4E* treatment for these cell lines (Fig. 3B, Supplementary Fig. S5). Taken together, these results indicate that silencing *eIF4E* by ECO/siRNA nanoparticles can overcome chemoresistance and inhibit EMT or invasion of PCa cells with a mesenchymal phenotype (PC3 and DU145-DR), rather than an epithelial phenotype (DU145 and PC3-DR).

In the context of tumor plasticity, these results have important implications in the treatment planning for PCa, which is highly heterogeneous. As a result of this heterogeneity, the bulk tumor might include a diverse collection of cells harboring distinct molecular signatures with different characteristics and thus result in differential levels of sensitivity to treatment [11]. In the present study, *eIF4E* expression is high in cells undergoing EMT, irrespective of drug resistance. Silencing *eIF4E* by ECO/siRNA nanoparticles can overcome chemoresistance and inhibit EMT or migration in PCa cells with a mesenchymal phenotype (PC3 and DU145-DR), rather than an epithelial phenotype (DU145 and PC3-DR). These results have important implications on targeting *eIF4E* for the treatment of PCa with EMT features.

## DECLARATIONS

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

### CONSENT FOR PUBLICATION

Not applicable.

### AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

### COMPETING INTERESTS

ZRL is a co-founder of Cleveland Theranostics, LLC, a startup company focused on the development of multifunctional pH-sensitive amino lipids for gene therapy.

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## AUTHORS' CONTRIBUTIONS

XL and ZRL contributed substantially to the conception and design of this study. XL participated in experimental execution of all aspects of this study. DS, NA, AS prepared the carrier formulated nanoparticles. YZ participated in the characterization of nanoparticles. XL and AMV analyzed the data. XL, AMV and ZRL wrote and edited the manuscript. All authors read and approved the final manuscript.

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