IECC 2026 Conference
The 5th International Electronic Conference on Cancers
10–12 June 2026 | Online
MDPI

CRISPR/Cas9-based EPS8 knockout and its effect on drug resistance against gemcitabine on pancreatic cancer cells

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INTRODUCTION & AIM

Pancreatic cancer is one of the deadliest malignancies with poor prognosis and high resistance to chemotherapy. Gemcitabine, a deoxycytidine nucleoside analog, remains one of the standard first-line chemotherapeutic agents for pancreatic cancer treatment. After intracellular uptake, gemcitabine is phosphorylated into its active metabolites, which inhibit DNA synthesis by incorporation into newly synthesized DNA strands and suppression of DNA polymerase activity, ultimately leading to inhibition of tumor cell proliferation. However, both intrinsic and acquired resistance mechanisms substantially reduce its clinical efficacy and contribute to treatment failure (1,2).

EPS8 (Epidermal Growth Factor Receptor Pathway Substrate 8) is a signaling adaptor protein involved in cell proliferation, migration, cytoskeletal remodeling, and oncogenic signaling pathways. It functions as a downstream effector of receptor tyrosine kinase signaling and plays an important role in regulating actin dynamics, cellular motility, and signal transduction. Aberrant EPS8 expression has been associated with tumor progression, metastasis, and poor therapeutic response in multiple cancers (3,4). In addition, increased EPS8 expression has been linked to activation of pathways involved in cell survival and adaptation, suggesting a potential role in tumor aggressiveness and chemoresistance.

Evidence suggests that dysregulated EPS8-mediated signaling contributes to chemoresistance through activation of survival pathways, epithelial–mesenchymal transition (EMT), and enhanced tumor cell adaptation mechanisms (5).

CRISPR/Cas9 genome editing provides an efficient strategy for functional investigation of cancer-associated genes. Therefore, this study aimed to investigate whether EPS8 knockout could reduce gemcitabine resistance in pancreatic cancer cells (6).

METHOD

Bioinformatic Analysis: Gene expression profiles from publicly available pancreatic cancer datasets were analyzed using R software and differential expression analysis approaches. EPS8 expression levels in tumor and normal samples were evaluated, and EPS8 was selected based on its significant overexpression and potential role in tumor progression and chemoresistance.

sgRNA Design and Plasmid Construction: Two sgRNAs targeting the EPS8 genomic sequence were designed and cloned into a CRISPR/Cas9 expression vector for targeted gene disruption.

Cell Line Selection and Culture: Human pancreatic cancer cell lines (Aspc-1 and Panc-1) were screened for basal EPS8 expression, and the cell line exhibiting the highest expression level was selected for subsequent experiments and maintained under standard culture conditions.

CRISPR/Cas9 Knockout: The recombinant CRISPR/Cas9 construct was introduced into the selected pancreatic cancer cell line through transfection. Following transfection, puromycin selection was performed to enrich successfully transfected cells and isolate single-cell clones.

Validation: Gene disruption was initially confirmed by genomic PCR and Sanger sequencing. EPS8 expression levels in edited clones were subsequently evaluated using RT-qPCR and western blot analysis.

Drug Sensitivity Assay: To investigate the effect of EPS8 knockout on gemcitabine resistance, wild-type cells and edited clones were treated with different concentrations of gemcitabine, and cell viability was assessed using the MTS assay.

RESULTS & DISCUSSION

1. Bioinformatic Analysis
Bioinformatic analysis of pancreatic cancer datasets identified EPS8 as a significantly overexpressed gene associated with poor prognosis. Further analysis of gemcitabine-resistant datasets demonstrated elevated EPS8 expression in resistant models, suggesting a potential role for EPS8 in chemoresistance (Figure 1).

2. EPS8 Expression Validation
Expression analysis confirmed elevated EPS8 levels in pancreatic cancer cell lines. The cell line exhibiting the highest basal EPS8 expression was selected for subsequent genome editing experiments (Figure 2).

3. CRISPR/Cas9-Mediated EPS8 Knockout
Successful disruption of EPS8 was confirmed by genomic PCR and Sanger sequencing, demonstrating alterations at the target locus. Reduced EPS8 expression in edited clones was further validated by RT-qPCR and western blot analysis (Figure 3).

4. Effect of EPS8 Knockout on Gemcitabine Sensitivity
Edited clones exhibited decreased cell viability following gemcitabine treatment compared with wild-type controls. Drug-response analysis demonstrated enhanced sensitivity to gemcitabine after EPS8 knockout, supporting a potential role for EPS8 in mediating chemoresistance in pancreatic cancer cells (Figure 4).

5. Morphological Assessment
Edited clones exhibited marked morphological alterations compared with wild-type cells. Noticeable changes in cellular morphology and growth characteristics were observed following EPS8 knockout, suggesting that disruption of EPS8 may influence the phenotypic properties of pancreatic cancer cells.

Discussion: EPS8 appears to play an important role in regulating gemcitabine response in pancreatic cancer cells.
Knockout of EPS8 increased drug sensitivity, suggesting its involvement in chemoresistance mechanisms.
The observed morphological changes further support its functional role in maintaining malignant cell behavior.
These findings highlight EPS8 as a potential therapeutic target in pancreatic cancer.
Further studies are required to clarify the underlying molecular mechanisms.

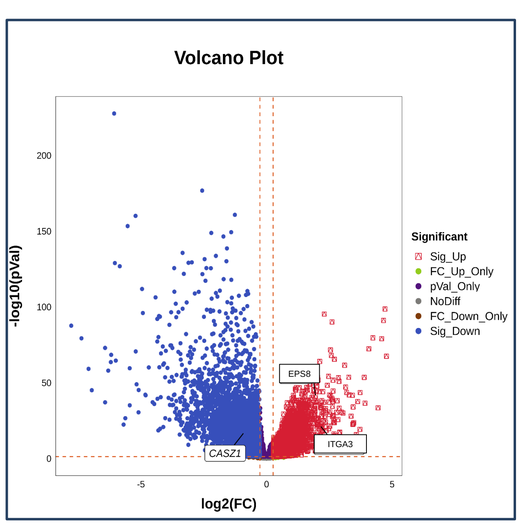

Figure 1. Volcano plot of differentially expressed genes in pancreatic cancer (TCGA dataset), highlighting significantly upregulated genes in red and downregulated genes in blue, with EPS8 identified as a prominent upregulated candidate potentially involved in tumor progression and drug resistance.

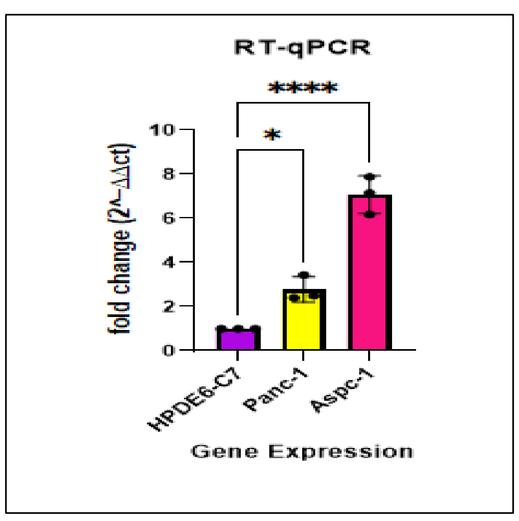

Figure 2. Relative expression analysis of EPS8 in pancreatic cancer cell lines compared with non-tumorigenic HPDE6-C7 cells using RT-qPCR. Data are presented as mean ± standard deviation from three independent experiments performed in triplicate. Statistical analysis was performed using GraphPad Prism, and *P < 0.05 was considered statistically significant.

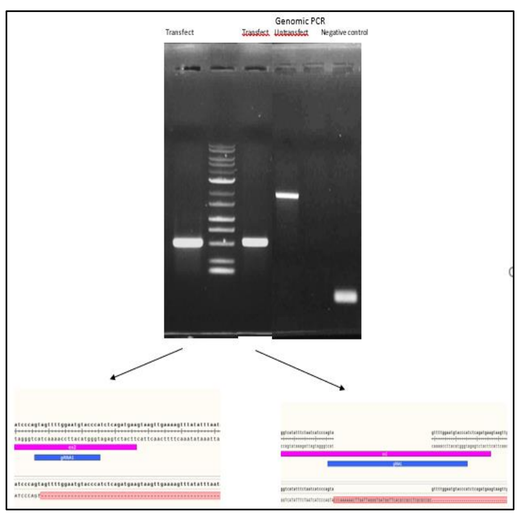

Figure 3. Confirmation of EPS8 knockout in edited pancreatic cancer cells using genomic PCR and Sanger sequencing. Genomic PCR analysis demonstrated successful amplification of target loci alterations, while Sanger sequencing confirmed indel formation at the EPS8 target site, indicating effective CRISPR/Cas9-mediated gene disruption.

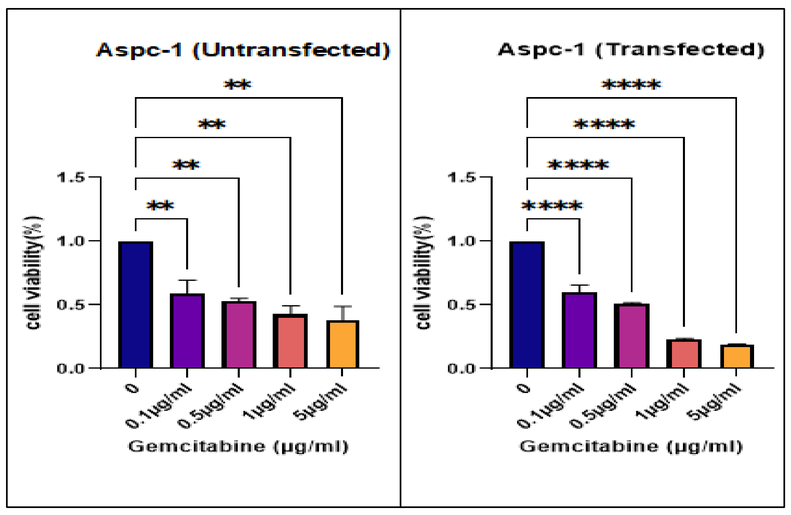

Figure 4. Evaluation of gemcitabine sensitivity in AsPC-1 cells following EPS8 knockout. Cells were treated with increasing concentrations of gemcitabine for 48 hours, and cell viability was determined using the MTS assay. IC50 values were estimated using nonlinear regression analysis in GraphPad Prism, revealing a shift in drug response after EPS8 depletion. Data are shown as mean ± standard deviation from three independent experiments performed in triplicate. Statistical significance was defined as *P < 0.05.**

CONCLUSIONS

EPS8 knockout significantly increased the sensitivity of pancreatic ductal adenocarcinoma (PDAC) cells to gemcitabine, indicating its potential role in mediating chemoresistance.
These results suggest that targeting EPS8 may enhance the therapeutic efficacy of standard chemotherapy in pancreatic cancer. Further studies in additional experimental models are required to validate these findings.

FUTURE WORK/ REFERENCES/ACKNOWLEDGMENT

Future Work
Further validation of EPS8 function in additional pancreatic cancer models is required, including in vivo studies and mechanistic investigation of downstream signaling pathways involved in gemcitabine resistance. Evaluation of EPS8 as a potential therapeutic target in combination with standard chemotherapy is also warranted.

References

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