TITLE

A Tumor-penetrating Recombinant Protein anti-EGFR-iRGD Enchance Efficacy of Paclitaxel in 3D Multicellular Spheroids and Gastric Cancer in vivo

Authors: Huizi Sha, Rutian Li, Jia Wei, Xinyu Bian, Qin Liu, Weiwei Kong, Xiaoping Qian, Wenjing Hu, Baorui Liu

Hospital: The Comprehensive Cancer Center of Drum-Tower Hospital, Medical School of Nanjing University & Clinical Cancer Institute of Nanjing University, Nanjing, China

OBJECTIVES

Human tumors, including gastric cancer, frequently express high levels of epidermal growth factor receptors (EGFRs), which are associated with a poor prognosis. Targeted delivery of anticancer drugs to cancerous tissues shows potential in sparing unaffected tissues. However, it has been a major challenge for drug penetration in solid tumor tissues due to the complicated tumor microenvironment. To improve drugs' (including PTX) penetration ability, recombinant protein anti-EGFR-iRGD was purified and examined.

METHODS

Recombinant protein anti-EGFR-iRGD consisting of an anti-EGFR VHH (the variable domain from the heavy chain of the antibody) fused to iRGD, a tumor-specific binding peptide with high permeability were expressed in *E. coli* BL21 (DE3) and purified by nickel-nitrilotriacetic acid affinity chromatography. We use tumor cell lines (2D), multicellular spheroids (3D), and mice to analyze the antitumor activity of recombinant protein. To investigate the ability of anti-EGFR-iRGD to improve other drugs (e.g. PTX) penetrating into tumor, we used multicellular spheroids and mice.

Graphs and tables

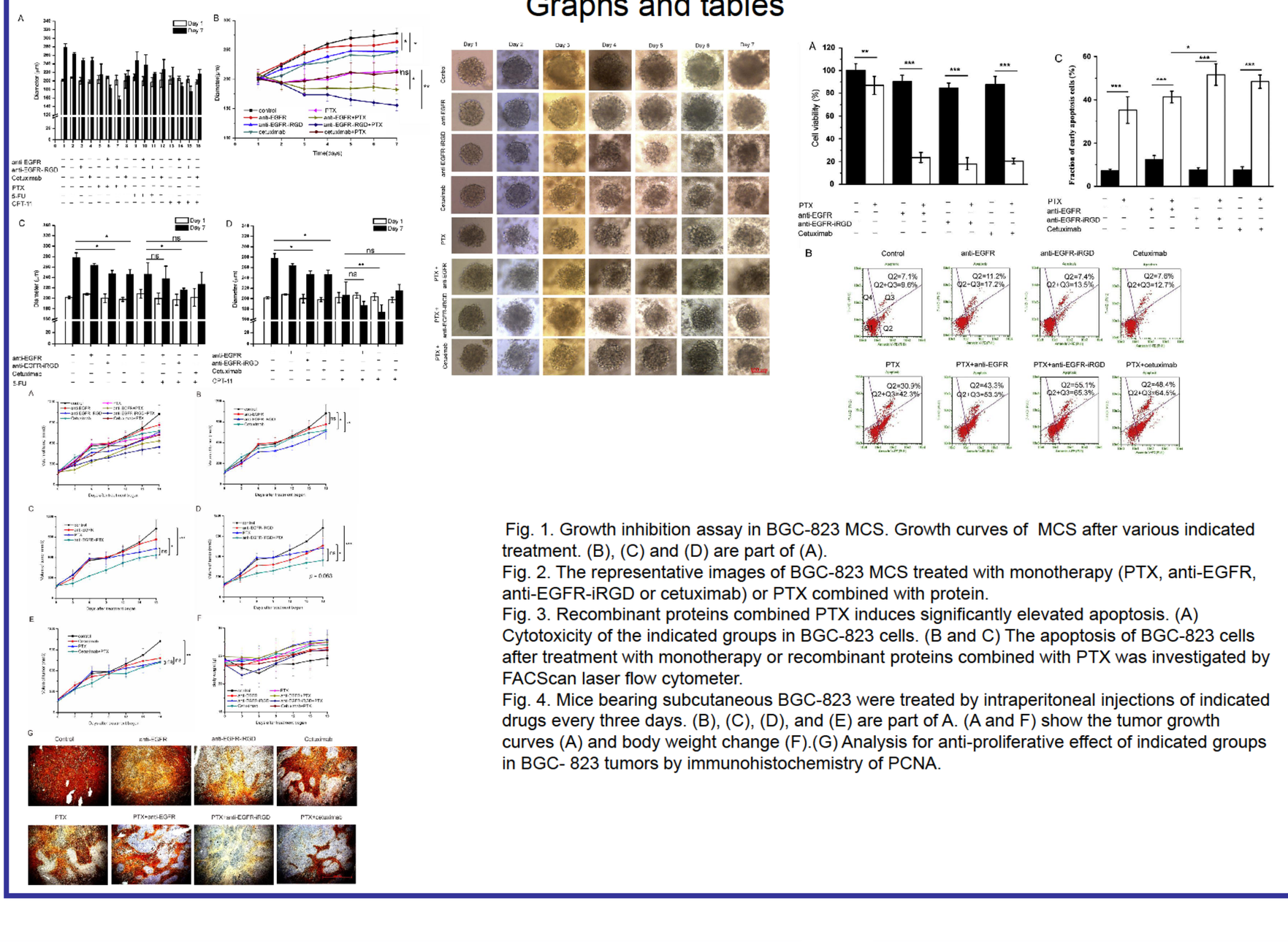

Fig. 1. Growth inhibition assay in BGC-823 MCS. Growth curves of MCS after various indicated treatment. (B), (C) and (D) are part of (A).
Fig. 2. The representative images of BGC-823 MCS treated with monotherapy (PTX, anti-EGFR, anti-EGFR-iRGD or cetuximab) or PTX combined with protein.
Fig. 3. Recombinant proteins combined PTX induces significantly elevated apoptosis. (A) Cytotoxicity of the indicated groups in BGC-823 cells. (B and C) The apoptosis of BGC-823 cells after treatment with monotherapy or recombinant proteins combined with PTX was investigated by FACScan laser flow cytometer.
Fig. 4. Mice bearing subcutaneous BGC-823 were treated by intraperitoneal injections of indicated drugs every three days. (B), (C), (D), and (E) are part of A. (A and F) show the tumor growth curves (A) and body weight change (F).(G) Analysis for anti-proliferative effect of indicated groups in BGC- 823 tumors by immunohistochemistry of PCNA.

RESULTS

Recombinant protein anti-EGFR-iRGD consisting of an anti-EGFR VHH (the variable domain from the heavy chain of the antibody) fused to iRGD, a tumor-specific binding peptide with high permeability were expressed in *E. coli* BL21 (DE3) and purified by nickel-nitrilotriacetic acid affinity chromatography. We use tumor cell lines (2D), multicellular spheroids (3D), and mice to analyze the antitumor activity of recombinant protein. To investigate the ability of anti-EGFR-iRGD to improve other drugs (e.g. PTX) penetrating into tumor, we used multicellular spheroids and mice.

CONCLUSIONS

Our results provide impetus for further studies for potentially using iRGD based fusion protein anti-EGFR-iRGD with standard cytotoxic treatment regimens for enhancing therapy of gastric cancer patients.

References

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