In vivo therapy of osteosarcoma using anion transporters based supramolecular drugs

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Disclosures: Z. Zheng: None, X. Wei: None, C. Lang: None, F. Zhao: None, J. Chen: None

INTRODUCTION: Osteosarcoma represents a serious clinical challenge due to its widespread genomic alterations, tendency for drug resistance and distant metastasis. In recent years, small-molecule based anion transporter have emerged as innovative and promising therapeutic compound with various biomedical applications. However, due to a lack of efficient delivery methods, previous research mainly focused on in vitro studies of anion transporters using lipid bilayers and cell models. There is an urgent need to develop a novel drug delivery system targeting tumors in order to expand the in vivo application prospects of small molecule ion transporters.

METHODS: Bimodal ion transporters used in this study were based on thiourea groups, and were synthesized by reacting bis(2-aminoethyl) ether with corresponding isothiocyanates (PTU, TFPTU, BTFPTU). We evaluated the diameters of LUVs of egg yolk phosphatidylcholine (EYPC) with small-molecule based anion transporter. The ^{1}H NMR spectrum and 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) assay were used to characterize the anion transporter. CCK-8, clone formation assay, EdU assay and flow cytometry were used to confirm cytotoxic effects of BTFPTU on osteosarcoma cells. RNA sequencing was employed to assess whether BTFPTU triggers reprogramming of osteosarcoma cells. Through a co-assembly process, we have successfully prepared supramolecular drugs by loading anion transporters into targeting peptide functionalized liposomes. We constructed subcutaneous xenograft tumor and lung metastasis models to evaluate the in vivo targeting and therapeutic efficacy of the assemblies, as well as its regulatory effects on the tumor immune microenvironment. All animal experiments were approved by the ethics committee of Zhejiang University.

RESULTS SECTION: We synthesized three small ion transporters (PTU, TFPTU, BTFPTU) and identified BTFPTU as having the best tumor killing effect. BTFPTU triggers reprogramming of HOS cells and induced cell death through multiple pathways. These pathways included activation of endoplasmic reticulum stress, autophagy, apoptosis and cell cycle arrest. The self-assembled osteosarcoma targeting peptide-BTFPTU supramolecular liposomes (OTP-BP-L) inhibits the proliferation and migration of HOS cells in vitro. OTP-BP-L had good tumor-targeting ability and accumulated in tumor tissue. It exhibited significant therapeutic effects in osteosarcoma tumorigenesis and metastasis in vivo and didn't cause significant damage to heart, kidney, liver, lung, and spleen. OTP-BP-L was still effective in killing drug resistance cell lines of osteosarcoma (HOS-DDPR) and other tumors. OTP-BP-L treatment significantly promoted M1 ($CD86^+/F4/80^+$) polarization and inhibited M2 ($CD206^+/F4/80^+$) polarization of tumor-associated macrophages. OTP-BP-L also achieved its therapeutic effect by regulating the tumor immune microenvironment.

DISCUSSION: Through a co-assembly process, we have successfully prepared supramolecular drugs by loading anion transporters into osteosarcoma targeting peptide functionalized liposomes. The assemblies, OTP-BP-L, show excellent targeting and therapeutic effect towards osteosarcoma tumors and a strong ability to regulate the tumor immune microenvironment. To further apply OTP-BP-L in vivo, the retention time in tumor tissue and metabolic processes of OTP-BP-L will be the focus of our future research. This work not only demonstrated the biomedical value of small-molecule anion transporters in vivo, but also provided an innovative approach for the treatment of osteosarcoma.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Utilizing a self-assembled system, we have demonstrated for the first time that small-molecule anion transporters based supramolecular drugs are capable of killing osteosarcoma cells in vivo. We have also shown that, rather than solely relying on the previously reported caspase-dependent apoptotic pathway, osteosarcoma cell death was induced through multiple pathways.

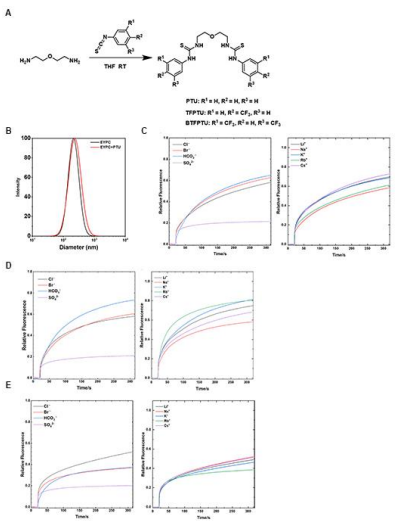

Fig.1 Synthetic procedures and transmembrane ion transport properties of PTU, TFPTU and BTFPTU. (A) Synthetic routes of PTU, TFPTU and BTFPTU. (B) Dynamic light scattering (DLS) measurements of large unilamellar vesicles (LUVs) made from blank EYPC and EYPC with anion transporter PTU. (C-E) Anionic and cationic selectivity of PTU, TFPTU and BTFPTU determined using the HPTS assays.

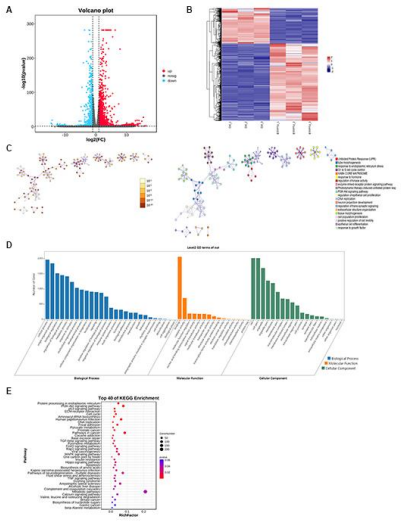

Fig.2 BTFPTU triggers transcriptional reprogramming of HOS cells. (A) Volcano plot of RNA sequencing (RNA-seq) analysis on HOS cells treated with either PBS or BTFPTU. (B) The differential gene clustering heatmap of RNA-seq analysis. (C) The network of enriched terms of RNA-seq analysis. The left figure was colored by P-value and terms containing more genes tended to have a more significant P-value. The right figure was colored by cluster ID and nodes share the same cluster were typically close to each other. (D) GO enrichment of RNA-seq analysis. (E) KEGG enrichment of RNA-seq analysis. $p < 0.05$ is defined as having a significant difference for comparison with the control group.

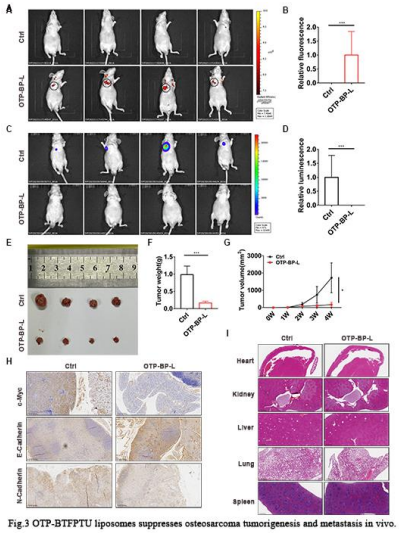

Fig.3 OTP-BTFPTU liposomes suppresses osteosarcoma tumorigenesis and metastasis in vivo. (A,B) Living fluorescence imaging of subcutaneous xenograft tumor model mice. (C,D) Living luminescence imaging of subcutaneous xenograft tumor model mice (E) Photographs of HOS derived xenograft model with OTP-BP-L treatment. (F) The average tumor weight in each group when the mice were sacrificed. (G) Tumor volume of different groups was measured after mice were injected with HOS cells. (H) The expression of c-Myc, E-cadherin and N-cadherin were determined by immunohistochemistry. (I) HE staining of heart, kidney, liver, lung and spleen which were obtained from different groups. The data represent the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 for a comparison with the control group or as indicated.