Euro Pharmaceutics 2018: Tumor microenvironment-responsive activation of nano delivery systems- Yu Kyoung Oh- Seoul National University.

Yu Kyoung Oh[1]

[1]Seoul National University, Republic of Korea

Abstract:

The tumour microenvironment has recently emerged as a new target of anticancer chemotherapy. Selective activation of anticancer chemotherapy in the tumor microenvironment would further reduce the toxicity of anticancer drugs toward normal tissues. Fibroblast activation protein (FAP) is known to be selectively overexpressed on cancer-associated fibroblasts (CAFs) in the tumor microenvironment. Here, we designed an anticancer chemotherapeutic system based on promelittin, a peptide toxin that is selectively converted from an inactive form to the pore-forming melittin upon cleavage by FAP in the tumor microenvironment.

We conjugated promelittin-containing FAP-cleavable sequences to pegylated phospholipids and anchored them to reduced graphene oxide (rGO) nanosheets. The resulting nanosheets, PL-rGO, were tested for haemolysis and used for doxorubicin delivery. In vitro cocultures and in vivo tumor growth (n = 5 mice per group) with tissue immune-staining were used to test the selective activation of anticancer chemotherapy by FAP expressed on CAFs.

Results:

FAP-specific hemolytic activity of PL-rGO was observed in cocultures of CAFs and HT29 cells but not in HT29 cells alone. Doxorubicin-loaded PL-rGO (Dox/PL-rGO) showed 3.4-fold greater cell-killing efficacy (compared with free Dox in the CAF/HT29 coculture system, effects that were not observed in HT29 cells alone). Intravenously administered Dox/PL-rGO reduced the growth of HT29 tumors more effectively than other treatments (Dox/PL-rGO: mean = 200.6 mm3, 95% confidence interval [CI] = 148.7 to 252.5 mm3; free Dox: mean = 697.0 mm3, 95% CI = 646.9 to 747.1 mm3, PL: mean = 565.0 mm3, 95% CI = 550.5 to 579.6 mm3; Dox/rGO: mean = 637.6 mm3, 95% CI = 619.5 to 655.7 mm3; PL-rGO: mean = 464.4 mm3, 95% CI = 433.0 to 495.8 mm3). Immunostaining of tumor tissues revealed that survival of CAFs and HT29 cells was lowest in the group treated with Dox/PL-rGO.

Conclusions:

The demonstration of selective activation of PL-rGO by FAP on CAFs suggests that PL-rGO may serve as a tumor microenvironment–responsive anticancer chemotherapy system. The tumor microenvironment has recently emerged as a new target of anticancer chemotherapy. This microenvironment—a complex system composed of many cell types, including fibroblasts, endothelial cells, and immune cells has been reported to contribute to cancer progression and dissemination. Among various cells in the tumor microenvironment, fibroblasts have received particular attention for microenvironment-targeted anticancer therapy

Methods:

- Preparation of PL-rGO Nanosheets
- Haemolysis Assay
- In Vitro Antitumor Efficacy Study Of Dox/PL-Rgo
- Assessment of In Vivo Antitumor Activity
- Statistical Analysis

Results:

Design and Characterization of Dox/PL-Rgo Nanosheets

Surfaces of rGO nanosheets were coated with PL and loaded with Dox. A possible working mechanism of Dox/PL-rGO. The promelittin moiety of Dox/PL-rGO nanosheets has a FAP-cleavable peptide sequence that is activated by FAP protease, which is overexpressed on CAFs in the tumor microenvironment, liberating the pore-forming peptide melittin.

FAP Expression-Dependent Hemolytic Activity of PL-Rgo Nanosheets

The expression levels of FAP differed between HT29 cells and CAFs. Flow cytometry revealed that 90.3% of CAFs were positive for FAP expression, whereas only 5.1% of HT29 cells were FAP-positive.

Cellular Uptake of PL-Rgo Nanosheets in the Presence of FAP-Positive CAFs

To demonstrate enhanced cellular uptake of PL-rGO, we first established FAP knockdown CAFs by transfection with siFAP (18). Treatment of CAFs with siFAP decreased the mRNA levels of FAP (mean = 30.9%, 95% CI = 27.1% to 34.8%, $P < .001$) compared with control levels.

In Vitro Anticancer Efficacy of Dox/PL-Rgo Nanosheets

We tested whether the increased uptake of PL-rGO translates to enhanced efficacy of Dox-loaded PL-rGO in vitro. The anticancer activity of Dox/PL-rGO was enhanced in the CAF/HT29 cell coculture system but not in FAP-negative HT29 cells (Figure 5A), where anticancer activity did not statistically significantly differ among all groups tested (Dox: mean = 90.5%, 95% CI = 88.2% to 92.8%, P = .91; PL: mean = 93.8%, 95% CI = 93.1% to 94.5%, P = .81; Dox + rGO: mean = 91.5%, 95%

CI = 90.3% to 92.6%, P = .98; Dox/rGO: mean = 93.9%, 95% CI = 91.3% to 96.6%, P = .85; PL-rGO: mean = 91.3%, 95% CI = 90.5% to 92.1%, P = .92; Dox + PL-rGO: mean = 89.7%, 95% CI = 84.8% to 94.6%, P = .86).

Cytotoxicity of Free Dox and Dox/PL-Rgo Nanosheets in Normal Fibroblasts

The differential cytotoxicity of free Dox and Dox/PL-rGO was investigated in normal fibroblasts. Free Dox showed a concentration-dependent cytotoxic effect in normal fibroblasts, In contrast, Dox/PL-rGO nanosheets did not statistically significantly induce cytotoxicity in normal fibroblasts compared with control cells

In Vivo Tumor Tissue Accumulation and Penetration of PL-Rgo Nanosheets

We next evaluated the tumor distribution of DSPE-PEG5000-Cy5.5 lipid-labeled rGO and PL-rGO following in vivo administration. At 24 hours postdose (Figure 6A), the accumulation of fluorescence at tumor sites was greater in mice treated with PL-rGO than in those treated with rGO.

In Vivo Toxicity of Dox/PL-Rgo Nanosheets

The in vivo toxicity of Dox/PL-rGO nanosheets was evaluated after a single injection or three repeated injections. Compared with untreated group, there were no statistically significant alterations in blood urea nitrogen

Discussion:

This study demonstrated that PL-rGO is activated by FAP expressed on CAFs in the tumor microenvironment and is capable of enhancing the cellular delivery and antitumor efficacy of Dox. PL, containing an FAP-cleavable peptide sequence, exerted an RBC lysis action following treatment with FAP, but not MMP.