Mesenchymal Stem Cell-Based Targeted Combined Therapy for Lung Cancer Management

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Background

- Lung cancer is the leading cancer killer in both men and women, accounting for ~25% of all cancer deaths.[1]
- Since a large proportion of patients (57%) are diagnosed with metastatic disease, the five-year relative survival rate is only 6%, resulting in a greater than 50% mortality rate within a year of diagnosis.[1]
- Current chemotherapeutic approaches for lung cancer extend life by only a few months compared with best supportive care in lung cancer.[2]
- Poor availability of chemotherapeutics in deep-seated and metastatic cancers results in the development of drug resistance and failed therapeutic outcomes.[3]
- Tumor-targeted delivery can enhance therapeutic efficacy while minimizing the nonspecific toxicity of anticancer drugs.
- Furthermore, combination therapies targeting different key signaling pathways are likely needed to overcome tumor resistance and achieve durable clinical responses.[4]

Purpose

The proposed research aims to advance mesenchymal stem cells (MSCs) as a safe and efficient drug delivery platform for tumor-targeted delivery of paclitaxel and entinostat combination to treat the patient-derived xenograft (PDX) model of non-small cell lung cancer (NSCLC).

Methods

- **Determination of synergistic potential:**
  - ✓ A549 cells were treated with cells various concentrations of paclitaxel, entinostat, and their combinations.
  - ✓ The cell viability was determined via MTT assay.
  - ✓ The combination interactions between free paclitaxel and entinostat were evaluated by synergy scoring models using Combenefit® software.
- **Generation of azide-labeled MSCs (MSC-Az):**
  - ✓ MSCs were cultured in N-azidoacetylmannosamine-tetraacylated (Ac_4ManNAz) supplemented MSC growth medium for three days.
  - ✓ Presence of azide groups on MSC surface was visualized by incubating MSC-Az with dibenzocyclooctyne (DBCO)-TAMRA dye followed by fluorescence microscopy.
  - ✓ Effect of azide sugar concentration: Flow cytometry
- **Preparation of DBCO-functionalized nanoparticles:** Paclitaxel and entinostat individually loaded DBCO-functionalized poly(DL-lactide-co-glycolide) nanoparticles were formulated by solvent evaporation technique.[5]
- **Nanoparticles characterization:**
  - ✓ Hydrodynamic diameter: dynamic light scattering
  - ✓ Surface charge: dynamic light scattering
  - ✓ Drug loading: HPLC
- **Drug loading of MSC-Az:** Nanoparticles loaded MSCs (nano-MSCs) were prepared by incubating MSC-Az with nanoparticles for 4 h at 37°C.[6]
- **Generation of PDX tumor model:** Surgical implantation of cancerous tissue from a patient's tumor into an immune-deficient mouse directly.

Central Hypothesis

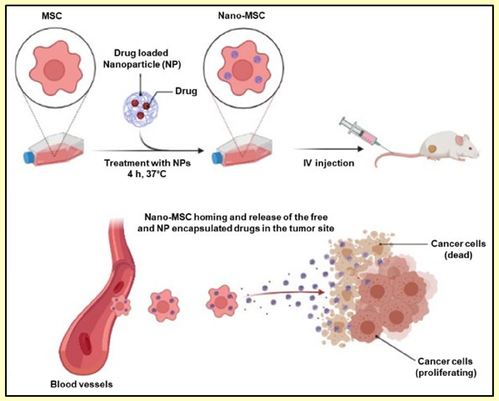

Figure 1. Schematic representation of nano-MSC-mediated tumor-targeted drug delivery.

Results

Synergistic killing of A549 cells

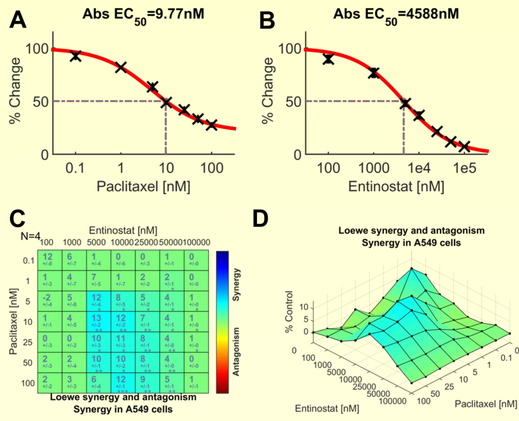

Figure 2. Synergy analysis by the Loewe model for paclitaxel and entinostat combination using Combenefit® software. (A) Paclitaxel dose-response curve, (B) Entinostat dose-response curve, (C) Synergy and antagonism distribution matrix, and (D) Synergy and antagonism surface (n = 6).

Paclitaxel and entinostat combination suppressed HDAC activity

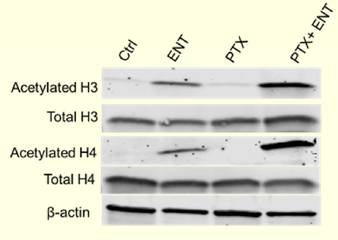

Figure 3. Combination therapy inhibits HDAC activity in A549 cells. Western blotting analysis of acetylated histones and total histones. Ctrl: control, ENT: entinostat, PTX: paclitaxel.

Results

Characterization of nanoparticles

Formulation	Particle size (nm)	Surface charge (mV)	Drug loading (% w/w%)
PTX NP	282 ± 5	-18.4 ± 2.5	18.1 ± 1.9
ENT NP	276 ± 4	-17.8 ± 0.5	14.5 ± 1.5

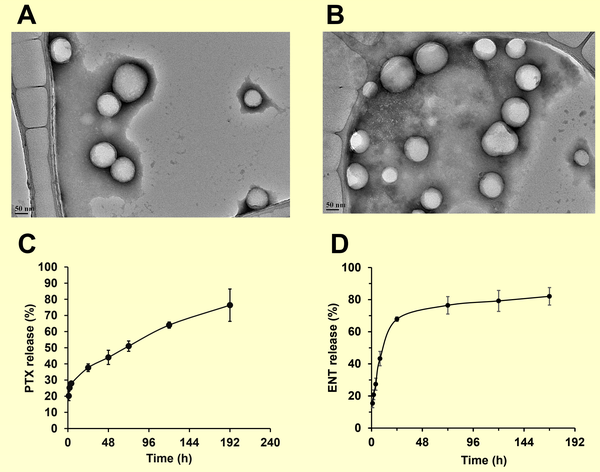

Figure 4. Characterization of DBCO-functionalized, drug-loaded nanoparticles. (A) A representative TEM image of PTX NPs (A) and ENT NPs (B). *In vitro* release profile of PTX NPs (C) and ENT NPs (D) from nanoparticles in complete cell culture medium supplemented with 10% (w/v) Captisol® at 37°C. Data shows mean ± SD (n = 4).

Preparation and characterization of nano-MSCs

PTX-loading of nano-MSCs: 55 µg/million of MSCs
ENT-loading of nano-MSCs: 48 µg/million of MSCs

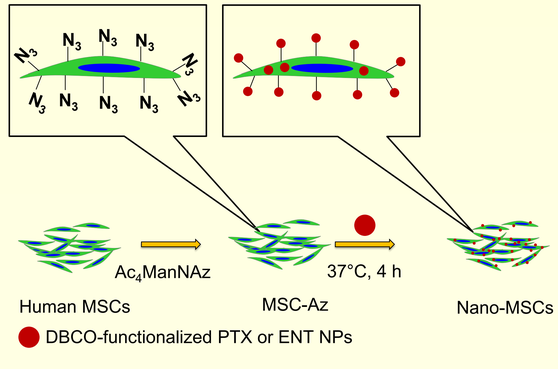

Figure 5. Schematic representation of azide-labeled MSCs as drug delivery carriers.

Results

Generation of PDX tumor model

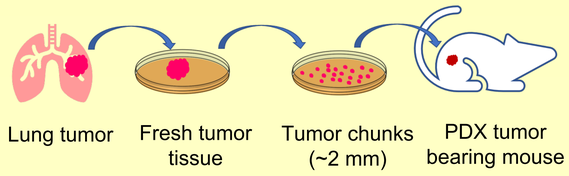

Figure 6. Schematic representation of PDX mouse model generation.

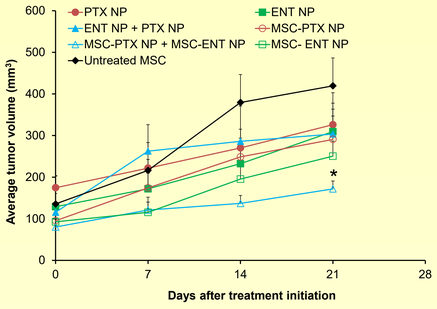

Figure 7. Antitumor efficacy of nano-MSCs. Mice were IV injected weekly with various treatments equivalent to 5 mg/kg of drug. Treatment of nano-MSC showed the minimum tumor growth. (*p < 0.05, n = 8). Data represent mean ± SEM.

Conclusions

- The HDAC inhibition by entinostat was synergistic with paclitaxel at multiple combinations of concentrations.
- Drug-loaded PLGA nanoparticles were spherical and exhibited high drug loading.
- MSC-Az incubated with DBCO-functionalized nanoparticles showed higher drug loading and retention.
- Nanoparticle conjugation on azide-labeled MSCs didn't affect their short-term and long-term viability as well as migration potential.
- Our preliminary in vivo data exhibited promising anticancer effects of combination treatment against the PDX tumor model.

References

1. Society AC. Facts & Figures 2020. Atlanta, Ga: American Cancer Society, 2020.
2. Clegg A, Scott DA, Sidhu M, Hewitson P, Waugh N. A rapid and systematic review of the clinical effectiveness and cost-effectiveness of paclitaxel, docetaxel, gemcitabine and vinorelbine in non-small-cell lung cancer. Health technology assessment (Winchester, England). 2001;5(32):1-195.
3. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, Sarkar S. Drug resistance in cancer: an overview. Cancers. 2014;6(3):1769-92.
4. Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, Yeger H. Combination therapy in combating cancer. Oncotarget. 2017;8(23):38022-43.
5. Layek B, Sadhukha T, Panyam J, Prabha S. Nano-Engineered Mesenchymal Stem Cells Increase Therapeutic Efficacy of Anticancer Drug Through True Active Tumor Targeting. Molecular Cancer Therapeutics. 2018;17(6):1196-206.
6. Layek B, Sehgal D, Argenta PA, Panyam J, Prabha S. Nanoengineering of Mesenchymal Stem Cells via Surface Modification for Efficient Cancer Therapy. Advanced Therapeutics. 2(9) (2019) 1900043.

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