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Investigation of CXCR4 Chemokine Receptor Involvement in the Uptake and Penetration of siRNA Nanoparticles in Pancreatic Cancer Cell Lines and 3D Tumor Spheroids

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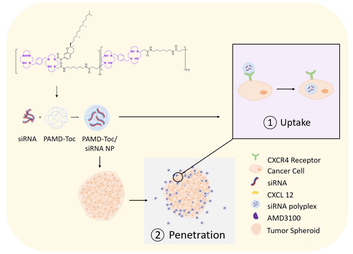
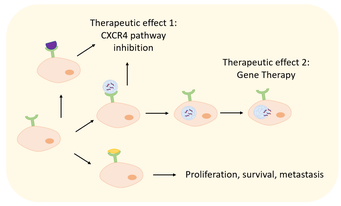
ABSTRACT

With the urgency of developing novel therapies for pancreatic cancer, the Oupický lab designed a polymer (PAMD-Toc) that incorporates AMD3100, an antagonist of the most widely expressed chemokine receptor in cancer cells, CXCR4. This study seeks to determine if the CXCR4 receptor is involved in the uptake and penetration of siRNA-carrying PAMD-Toc nanoparticles into pancreatic cancer cells and tumors. Findings suggest that the presence of CXCR4 increases the efficacy of PAMD-Toc nanoparticle uptake and tumor penetration, which makes PAMD-Toc nanoparticles a promising siRNA delivery vector for pancreatic cancer gene therapy.



Introduction

Pancreatic cancer is the third leading cause of cancer-related deaths in the United States. Over half of pancreatic cancer patients are diagnosed after the cancer has metastasized to distant tissues at which the five-year survival rate is 3%. Consequently, the development of therapies are urgent. The most widely expressed chemokine receptor in human cancer cells, the CXCR4 receptor and its ligand, CXCL12, promotes pancreatic cancer survival, proliferation, and metastasis, so the CXCR4/CXCL12 axis can be approached as a target for drug discovery. An ideal delivery vector would protect the siRNA drug while facilitating efficient penetration and uptake in the tumor and cancer cell respectively by interacting with the CXCR4 pathway. Our lab has synthesized the polymer, PAMD-Toc from AMD3100, HMBA, and tocopherol. AMD3100 is an FDA-approved small molecule drug that functions as a CXCR4 antagonist. While the specificity and antagonism of AMD3100 for the CXCR4 receptor has been established, whether the CXCR4 receptor plays a role in 1) uptaking PAMD-Toc/siRNA nanoparticles and 2) in deep penetration of pancreatic cancer tumors is yet to be understood.



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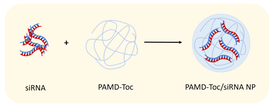
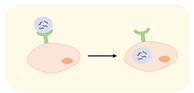
2. Wang Y, Li J, Chen Y, Oupický D. Balancing polymer hydrophobicity for ligand presentation and siRNA delivery in dual function CXCR4 inhibiting polyplexes. *Biomater Sci*. 2015;3(7):1114-1123.
3. Ying Xie, Yu Hang, Yazhe Wang, Richard Sleightholm, Dipakkumar R. Prajapati, Johannes Bader, Ao Yu, Weimin Tang, Lee Jaramillo, Jing Li, Rakesh K. Singh, and David Oupický. *ACS Nano* **2020** *14* (1), 255-271

Methods

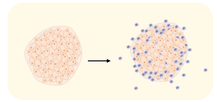
1. PAMD-Toc polyplexes were made by mixing varied weight to weight ratios of PAMD-Toc to siRNA. The particles' sizes and zeta potentials were characterized by the dynamic light scatterer (DLS), and the weight to weight ratio of PAMD-Toc to siRNA for optimal particles was determined.



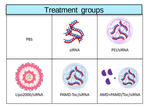


2. KPC8060, a murine pancreatic cancer cell line, and Panc-1, a human pancreatic cancer cell line, were treated with the PAMD-Toc/siRNA formulation, and as a control, cells were pretreated with AMD3100 to block the CXCR4 receptor and subsequently treated with PAMD-Toc/siRNA. cellular uptake was evaluated using flow cytometry and confocal microscopy.

3. To better simulate the complexities of drug delivery to a 3D tumor, KPC8060 and tumor spheroids were grown and treated with PAMD-Toc/siRNA formulation and tumor penetration was evaluated using confocal microscopy.



Results



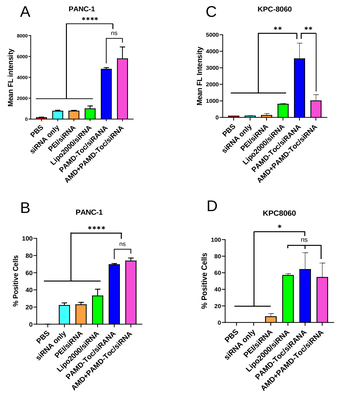


Figure 1. (a,b) Cellular uptake in Panc-1 cells. The Panc-1 cells showed no significant difference in mean fluorescence intensity between AMD3100 pretreated cells and non-pretreated cells. The percent of Panc-1 cells positive for fluorescence was also not significantly different between pretreated and non-pretreated cells. (c,d) Cellular uptake in KPC8060 cells. The KPC8060 cells showed significant difference in mean fluorescence intensity between the pretreated cells and non-pretreated cells. However, the percent of KPC8060 cells positive for fluorescence was not significantly different between pretreated and non-pretreated cells. One-way ANOVA with Turkey's multiple-comparison test was used. Significance is indicated as *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

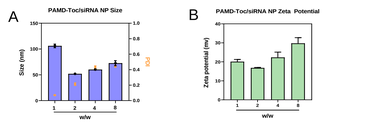


Figure 2. (a) DLS size characterization of PAMD-Toc/siRNA nanoparticles. All nanoparticles were within the 20-200 nm range. The 2:1 and 4:1 PAMD-TOC to siRNA weight to weight ratio yielded nanoparticles within the 10-70 nm range, ideal for capillary penetration. Particles made from 2:1 weight to weight ratio displayed lower PDI than 4:1.

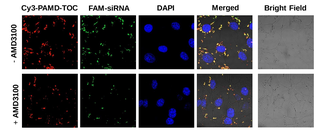


Figure 3. Confocal microscopy images showing greater PAMD-Toc/siRNA nanoparticle uptake in KPC8060 cells that have not been pre-treated with AMD3100 (-) compared to KPC8060 cells that have been pre-treated with AMD3100 (+).

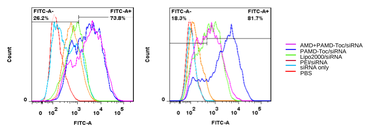


Figure 4. A comparison of fluorescence intensity distribution of the Panc-1 cells (left) and KPC8060 cells (right) shows a clear decrease in mean fluorescence intensity of KPC8060 cells that were pretreated with AMD3100 from the cells that were not pretreated, but the Panc-1 cells did not show a difference between cells that were pretreated and non pretreated with AMD3100.

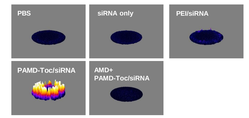


Figure 5. Confocal microscopy images comparing penetration of the treatment groups in KPC8060 tumor spheroids. When tumor spheroids were pretreated with AMD3100 to block the CXCR4 receptor, siRNA penetration was decreased significantly, indicating that siRNA penetration was most dependent on the CXCR4 receptor.

Discussion

siRNA cellular uptake was decreased significantly when KPC8060 cells were pretreated with AMD3100, which supports that CXCR4 is involved in the siRNA-carrying PAMD-Toc nanoparticle uptake. Confocal microscopy of KPC8060 cells corroborated that there was decreased uptake of PAMD-Toc/siRNA nanoparticles in the cells pretreated with AMD3100 than in non-pretreated cells. However, the % of cells positive for fluorescence was not significant between the pretreated and non-pretreated groups, indicating that CXCR4 enhances the efficacy of uptake, but is not the only variable involved in the uptake. Also, PAMD-Toc nanoparticles displayed greater efficiency of siRNA delivery than nanoparticles made of the widely-used polyethyleneimine (PEI) and Lipofectamine2000.

Due to Panc-1 cells exhibiting negligible CXCR4 surface expression in normoxic conditions, the AMD3100 pretreatment did not affect he siRNA cellular uptake in the Panc-1 cell line.

Confocal microscopy of KPC8060 tumor spheroids supports that CXCR4 promotes deeper penetration of PAMD-Toc nanoparticles into pancreatic cancer tumors.

Conclusion and Future Directions

With support for CXCR4 involvement in penetration and uptake in pancreatic cancer tumors and cells, siRNA nanoparticles incorporating AMD3100 via the polymer, PAMD-Toc show potential for a combined and targeted pancreatic therapy by not only the blocking of the CXCR4/CXCL12 axis, reducing cancer survival, proliferation, and metastasis, but also by targeting an overexpressed receptor for the purpose of deep tumor penetration and cellular uptake, allowing for a potent gene therapy. In further investigation, COLO357, a human metastatic pancreatic cancer line that expresses CXCR4 in normoxic conditions or Panc-1 cells in hypoxic conditions could be studied. With evidence to support that the CXCR4 receptor is involved in PAMD-Toc/siRNA nanoparticle penetration and uptake, future studies may also seek to characterize the specific mechanism by which it facilitates penetration and uptake.

Acknowledgements

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