利用魚精蛋白與脂質製備基因載體應用於 RNA 傳遞

李柔(5140)
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大綱

摘要

小分子干擾 RNA (small interfering RNA, siRNA)常用於 RNA 干擾(RNA interference, RNAi)作用，其可抑制或沉默(silencing)相同鹼基序列的 mRNA (messenger RNA)，阻止基因轉錄轉譯成蛋白質，利於治療基因缺陷所導致的疾病。由於 RNA 易被分解，故使用有助於 DNA 縮合(DNA condensation)的魚精蛋白(protamine)與 RNAi 干擾物包覆在三種不同型態的基因脂質載體，依序為 DNA 複合物(DNA-complex)、脂質奈米粒(lipid nanoparticles, LNP)與固體脂質奈米粒(solid lipid nanoparticles, SLNs)，測試其體內或體外的基因沉默能力。在使用魚精蛋白、脂質與 DNA 複合載體時，在魚精蛋白/核酸比在 0.6 時可組裝出 200 nm 和低多分散性的帶正電顆粒，對細胞不具顯著毒性，並具有 VEGFR-2 沉默能力；於組裝 LNP 時粒徑測得 435 nm，且對 pH 值具有敏感性的帶正電奈米載體，可誘導人類或小鼠細胞基因沉默率達 70%，證實陽離子細胞穿透胜肽(cell-penetrating peptides)的修飾有助於增強 LNP 的 B16F10 細胞內化(internalization)及基因沉默率；組裝 SLNs 時，測得粒徑介於 200-240 nm、帶正電奈米粒，並可保護 shRNA 免於酵素降解，細胞存活率可達 90%，展現 50%的基因沉默率。從三種不同型態的脂質與魚精蛋白基因載體，證實魚精蛋白可有效幫助脂質遞送 RNA 物質，以利於基因治療。

參考文獻

Alam, F., Al-Hilal, T. A., Chung, S. W., Park, J., Mahmud, F., Seo, D., Kim, H. S., Lee, D. S., & Byun, Y. (2015). Functionalized heparin-protamine based self-assembled nanocomplex for efficient anti-angiogenic therapy. *Journal of Controlled Release, 197*, 180-189.

Asai, T., Tsuzuku, T., Takahashi, S., Okamoto, A., Dewa, T., Nango, M., Hyodo, K., Ishihara, H., Kikuchi, H., & Oku, N. (2014). Cell-penetrating peptide-conjugated lipid nanoparticles for siRNA delivery. *Biochemical and Biophysical Research Communications, 444*(4), 599-604.

Burnett, J. C., Rossi, J. J., & Tiemann, K. (2011). Current progress of siRNA/shRNA therapeutics in clinical trials. *Biotechnology Journal, 6*(9), 1130-1146.

Hamoudi, M. C., Henry, E., Zerrouk, N., Scherman, D., Arnaud, P., Deprez, E., & Escriou, V. (2015). Enhancement of siRNA lipid-based vector stability and siRNA integrity in human serum with addition of anionic polymer adjuvant. *Journal of Drug Delivery Science and Technology, 26*, 1-9.

He, H. N., Sheng, J. Y., David, A. E., Kwon, Y. M., Zhang, J., Huang, Y. Z., Wang, J. X., & Yang, V. C. (2013). The use of low molecular weight protamine chemical chimera to enhance monomeric insulin intestinal absorption. *Biomaterials, 34*(31), 7733-7743.

Khoury, M., Louis-Plence, P., Escriou, V., Noel, D., Largeau, C., Cantos, C., Scherman, D., Jorgensen, C., & Apparailly, F. (2006). Efficient new cationic liposome formulation for systemic delivery of small interfering RNA silencing tumor necrosis factor alpha in experimental arthritis. *Arthritis and Rheumatism, 54*(6), 1867-1877.

Kwok, A., & Hart, S. L. (2011). Comparative structural and functional studies of nanoparticle formulations for DNA and siRNA delivery. *Nanomedicine-Nanotechnology Biology and Medicine, 7*(2), 210-219.

Li, S., & Huang, L. (1997). In vivo gene transfer via intravenous administration of cationic lipid-protamine-DNA (LPD) complexes. *Gene Therapy, 4*(9), 891-900.

Li, S., Rizzo, M. A., Bhattacharya, S., & Huang, L. (1998). Characterization of cationic lipid-protamine-DNA (LPD) complexes for intravenous gene delivery. *Gene Therapy, 5*(7), 930-937.

Li, S. D., & Huang, L. (2010). Stealth nanoparticles: High density but sheddable PEG is a key for tumor targeting. *Journal of Controlled Release, 145*(3), 178-181.

Nimesh, S. (2013). 12 - Protamine nanoparticles. In S. Nimesh (Ed.). *Gene Therapy* (pp. 237-258): Woodhead Publishing.

Potter, R., Hansen, L. T., & Gill, T. A. (2005). Inhibition of foodborne bacteria by native and modified protamine: Importance of electrostatic interactions. *International Journal of Food Microbiology, 103*(1), 23-34.

Prabhu, R., Garry, R. F., & Dash, S. (2006). Small interfering RNA targeted to stem-loop II of the 5 ' untranslated region effectively inhibits expression of six HCV genotypes. *Virology Journal, 3*. Retrieved November 2, 2006, from the World Wide Web: http://www.virologyj.com/content/3/1/100.

Scheicher, B., Schachner-Nedherer, A. L., & Zimmer, A. (2015). Protamine-oligonucleotide-nanoparticles: Recent advances in drug delivery and drug targeting. *European Journal of Pharmaceutical Sciences, 75*, 54-59.

Schlegel, A., Largeau, C., Bigey, P., Bessodes, M., Lebozec, K., Scherman, D., & Escriou, V. (2011). Anionic polymers for decreased toxicity and enhanced in vivo delivery of siRNA complexed with cationic liposomes. *Journal of Controlled Release, 152*(3), 393-401.

Temming, K., Schiffelers, R. M., Molema, G., & Kok, R. J. (2005). RGD-based strategies for selective delivery of therapeutics and imaging agents to the tumour vasculature. *Drug Resistance Updates, 8*(6), 381-402.

Torrecilla, J., del Pozo-Rodriguez, A., Apaolaza, P. S., Solinis, M. A., & Rodriguez-Gascon, A. (2015). Solid lipid nanoparticles as non-viral vector for the treatment of chronic hepatitis C by RNA interference. *International Journal of Pharmaceutics, 479*, 181-188.

Vader, P., Crielaard, B. J., van Dommelen, S. M., van der Meel, R., Storm, G., & Schiffelers, R. M. (2012). Targeted delivery of small interfering RNA to angiogenic endothelial cells with liposome-polycation-DNA particles. *Journal of Controlled Release, 160*(2), 211-216.