MEGAKARYOCYTIC MICROPARTICLES-MEDIATED NUCLEIC ACID DELIVERY FOR GENE THERAPY

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Cell-derived microparticles (MPs) are 0.1 to 1 micron extracellular vesicles (EVs), budding off cellular plasma membranes under stress or activation. MPs play an important role in cell-to-cell communication by transferring cargo from parent to target cells. Among circulating MPs, megakaryocyte-derived MPs (MkMPs) are the most abundant MPs in circulation (1). We have demonstrated that, *in vitro*, MkMPs specifically targeted and were taken up by human hematopoietic stem & progenitor cells (HSPCs) via fusion or endocytosis following specific receptor recognition (2). MkMPs transfer cargo to HSPCs and induce potent Mk differentiation of HSPCs in the absence of thrombopoietin (3). Here, we explored the capability of human MkMPs to transfer DNA and siRNA to HSPCs, and developed MkMP-based strategies for gene therapy. From our current protocol, we were able to achieve loading of plasmid DNA (pGFPns: encoding eGFP) into over 80% of MPs and functional delivery to HSPCs though co-culture to perform eGFP expression. DNA delivery efficiency were increased by optimized co-culture methods, chemically via fusogens to enhance membrane fusion, and physically to enhance contact between MPs and HSPCs. As a result, we were able to make possible that more than 20% of HSPCs express GFP. Functional RNA delivery was also studied by examining the impact of siR-MYB mediated *c-myb* silencing in enhancing Mk differentiation of $CD34^+$ cells. Our data demonstrate that MkMP-based delivery of siR-MYB to HSPCs enabled *c-myb* silencing that resulted in enhanced Mk differentiation beyond that of unloaded MkMPs, as assessed by a 29% increase of CD41 expression (an Mk marker), indicating functional siRNA delivery. To sum, functional pDNA and siRNA delivery to HSPCs via MkMPs demonstrate the potential of this delivery system for targeting the stem-cell compartment, suggesting that MkMPs constitute a potentially useful therapeutic delivery system for gene therapy, with applications in regenerative and transfusion medicine.

References:

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