Engineering Megakaryocytic Microparticles for Nucleic Acid Delivery and their Biological Effects to Hematopoietic Stem/Progenitor Cells

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ABSTRACT

Megakaryocytes (Mks) are large polyploid cells derived from hematopoietic stem and progenitor cells (HSPCs) in the bone marrow and give rise to platelets and megakaryocytic microparticles (MkMPs), the most abundant MPs in circulation. MkMPs can induce the differentiation of hematopoietic stem and progenitor cells (HSPCs) into functional Mks. In this study, we show that MkMPs target HSPCs with high specificity. Using fluorescent confocal microscopy and electron microscopy, we identify two mechanisms by which MkMPs deliver cargo to HSPCs: endocytosis, and specifically macropinocytosis and lipid raft-dependent endocytosis, and direct fusion of MkMPs into HSPCs. We show that HSPC uropods are the preferential site for MkMP binding, and that CD54 (ICAM-1), CD11b, CD18 and CD43, localized on HSPC uropods, are involved in MkMP binding to HSPCs. We also investigate the role of miRNAs from MkMP in MkMP-induced Mk differentiation of HSPCs. Our study identified the importance of three miRNAs, miR-486-5p, miR-92a-3p, and miR-22-3p, alone and in combination, in mediating Mk differentiation of HSPCs in the absence of thrombopoietin. Signaling pathways JNK, p38, and PI3K/mTOR are also identified as mediating the MkMP-induced Mk differentiation of HSPCs.

Since HSPCs are important target cells for gene therapy applications, we developed a nonviral system based on MkMPs for targeted delivery of plasmid DNA (pDNA) and small RNAs to HSPCs. With an optimized electroporation protocol, an average of 4200 plasmid copies per MP were loaded into MPs, thus enabling effective delivery of green fluorescent protein (GFP)–

encoding pDNA to HSPCs and HSPC nuclei, with up to 81% of the nuclei containing pDNA. Effective functional small interfering RNA (siRNA) and microRNA (miRNA) delivery were also demonstrated.

Finally, we examine the role of p53 in the Mk shear-stress response. We demonstrated that shear flow stimulates p53 acetylation and Caspase 9 activation, and demonstrated that shear-stimulated Caspase 9 activation and Mk particle biogenesis depend on transcription-independent p53-induced apoptosis (TIPA), but PS externalization is not.