THE HARTWELL FOUNDATION

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Microfluidic Platform to Isolate and Load Exosomes with Therapeutic Molecules

Nucleic acid-based gene therapy holds great promise as a life-saving treatment strategy to correct genetic diseases and disorders, including protein deficiencies or dysfunctional genes, without permanent manipulation of the genome. The use of noncoding RNA to target genes has made RNA-interference (RNAi) therapy a powerful gene-silencing intervention, particularly for cancer. However, despite significant progress in targeting solid tumors, the use of RNAi for blood cancers (leukemia, lymphoma and myeloma) is hindered because of RNA instability in blood (degraded by enzymes); the potential for RNA to trigger recipient immune rejection; and cellular barriers to RNA accessing molecular targets. Different delivery systems have been developed for RNAi gene therapy, but none are ideal; and certainly, none are as effective as the way natural living cells communicate with each other through the release of small, membrane bound vesicles (30 to 150 nm diameter), called exosomes. Depending upon their cell of origin, exosomes transport specific proteins, DNA, RNA and other effector molecules that when transferred and taken up by other cells may influence the phenotype of the recipient cells. Exosomes are present in most biological fluids including blood, saliva, and urine where notably, they seem to retain physical and functional stability, and if autologous do not provoke immune rejection. Notably, exosomes can cross cell membranes, including the blood brain barrier; and depending on exosome molecular composition, display an inherent ability to target certain tissues. The practical challenges are in overcoming at sufficient scale the notorious difficulty in exosome isolation and an effective way to achieve homogeneous loading of cargo. Based on her previous experience using electric current to open membrane pores or channels for controlled delivery of molecules into isolated mammalian cells (electroporation), Claire proposes to create a microfluidic system that will purify exosomes from recipient blood and subsequently, uniformly load desired RNAi into the exosome interior. The approach is based upon her hypothesis that contrary to expectation, electroporation of exosomes can be achieved at acceptably low operational voltages by exploiting a combination of exosomes bound to specific antibody-coated beads and local electric field enhancement on the dielectric bead surface, which will avoid the unsustainable electromechanical stresses that would otherwise occur at the estimated high voltage required for such small diameter vesicles. If successful, the ability to readily produce exosomes with encapsulated RNAi will have the potential to benefit the many children affected by cancer who are likely to respond to gene therapy and who may be able to avoid standard treatments and their related morbidities. Equally important, a practical methodology to isolate and load exosomes with therapeutic molecules will enable the strategic development of new applications in personalized precision medicine.