Molecular Therapy
Commentary

The evolving paradigm of extracellular vesicles in intercellular signaling and delivery of therapeutic RNAs

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Extracellular vesicles (EVs) are approximately 30- to 150-nM particles that are generated by cells and are thought to mediate the transfer of bioactive cargo.[1] Substantial evidence suggests that an under-appreciated role of EVs is the mediation of intercellular signaling via microRNAs (miRNAs).[1,2] However, the concept that miRNAs packaged into EVs mediate intercellular signaling has recently been called into question by Albanese and Chen et al.,[3] who report that miRNAs do not seem to be packaged appreciably into EVs and, furthermore, when they are, they have little to no effect on recipient cells. These observations call into question myriad publications over the last decade that suggested that EV-packaged miRNAs alter target cell function and play a role in disease development and even, for example, in reprogramming of cells toward an oncogenic phenotype.[2,4]

Interestingly, the findings from Albanese and Chen et al. are supported to a large extent by observations from others who actively try to engineer EVs to deliver therapeutic cargo: not only miRNAs, but also other small non-coding RNAs,[5] gene-encoding mRNAs,[6] zinc finger nucleases,[7,8] and even entire CRISPR/Cas systems.[9,10] Indeed, a common theme from these studies is that native EVs are not necessarily efficient in cargo delivery. Instead, significant engineering of the EV-producing cells or alteration of EVs post-production seems to be required for the functional packaging of therapeutically relevant RNA and other cargos and their release into recipient cells.[6–8] These manipulations may include fusion of RNA-binding domains to transmembrane proteins (e.g., CD63) and introduction of elements to promote endosomal escape.[2,3]

Collectively, the need for extensive manipulation of EV-producing cells and the observations by Albanese and Chen et al. suggest that native, unmanipulated EVs may not be the universally effective intercellular communicators that so many previously published studies have suggested them to be. Perhaps EVs represent agents mostly involved in expunging cell byproducts and/or in a metabolic recycling pathway rather than an intercellular signaling pathway after all?[7] Although these demonstrated EV roles of "taking out the trash" and "putting food on the table" are often disregarded as unimportant characteristics of EV biology in the literature, they are vital functions in the life of the cell. However, meagre cell fusion and cargo delivery abilities also do not necessarily preclude other roles in signaling. EVs may indeed serve as a scaffold for "kiss-and-run" interactions, in which the coordination of targeting and signaling ligands achieves more potent transduction than soluble ligands alone.[7]

If past is prologue, one thing is clear: our understanding of the inner workings of the cell and the role and function of EVs within it is merely a model that is constantly challenged and evolving. No doubt, future studies will provide greater insights, and invariably EVs may prove to be multifactorial elements involved in various aspects of cellular biology that can be taken advantage of for therapeutic gain. They are already emerging as the next-generation delivery agent that offer unique properties not imbued in lipid nanoparticles or viral vectors, ultimately resulting in a significant expansion of the gene therapy delivery tool kit.

DECLARATION OF INTERESTS

K.W.W. is an officer of the International Society for Extracellular Vesicles, advises NeuroDex and ShiftBio, and performs ad hoc private consulting in the EV space. K.M. has an interest in developing EV therapeutics to treat various human disease. U.S. patent 048440-749P01US on EV therapeutic technologies has been filed at the City of Hope where he has carried out his research.

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Worldwide use of factor IX Padua for hemophilia B gene therapy

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It is a bittersweet coincidence the Xue et al. published online their successful adeno-associated virus (AAV) gene therapy trial for Chinese men with hemophilia B (HB) utilizing the high-specific-activity factor IX (FIX) variant Padua in *Lancet Hematology*[1] almost on the same day that the co-discoverer of FIX-Padua,[2] Valder R. Arruda, passed away unexpectedly after a brief illness. Their report augments a growing list of promising gene therapy products for HB, all using FIX-Padua,[3,4] including at least three in phase 3. In part because of his early experiences practicing hematology in his native Brazil, Dr. Arruda was always interested in addressing the 80% of the worldwide hemophilia population outside of Western countries that have limited access to treatments;[5] thus, the phenotypic cure of at least 9 out of 10 non-Western HB subjects expressing FIX-Padua after gene therapy is a well-timed testament to his contributions to the field of gene therapy.

FIX-Padua was identified as a rare X-linked thrombophilia in a collaboration between Dr. Arruda's laboratory in Philadelphia and Dr. Paolo Simioni's team in Padua, Italy.[2] The two collaborators had met 10 years prior when they were both post-docs in Leiden, the Netherlands, which was typical of Dr. Arruda's ability to mix long-term friendships and science with colleagues from around the world. The proband had a spontaneous deep vein thrombosis and was found to have an 8-fold increase in FIX activity but a normal FIX antigen level. Dr. Arruda and his collaborators demonstrated that the single amino-acid substitution R338L in FIX-Padua resulted in an 8-fold increased specific activity compared with wild-type FIX. Further, they showed that the R338L substitution was present in the three affected members of the proband's family and was not present in two non-affected members.

The Arruda laboratory[6–8] and others[9] subsequently demonstrated that the hyperactivity of FIX-Padua could enhance HB gene therapy in preclinical models. This was especially exciting because of the growing recognition of the limitations of escalating AAV vector doses, especially the AAV-capsid cellular immune response.[10] The capsid immune response is AAV vector dose dependent and is most frequently clinically manifested as an asymptomatic hepatotoxicity but can result in the complete loss of transgene FIX.[10] The high-specific activity of FIX-Padua offered a strategy to achieve high FIX activity levels while maintaining lower vector doses—if safety considerations of using a variant transgene could be addressed.

To this end, the Arruda laboratory also demonstrated that FIX-Padua had comparable immunogenicity and thrombogenicity with wild-type FIX in experiments that spanned protein biochemical assays to large-animal studies,[6–8,11] including the highly compelling demonstration that a HB dog with a pre-existing antibody to wild-type FIX could be tolerized after liver-directed AAV gene therapy with FIX-Padua.[7] This result strongly supported the lack of immunogenicity concerns for FIX-Padua. These studies set the stage for the current widespread use of FIX-Padua in clinical gene therapy for HB.

Xue et al. treated 10 Chinese patients with HB with a novel AAV vector (BBM-H901). BBM-H901 has an engineered liver-tropic capsid with 98% identity with AAV6 and AAV1 and uses a synthesized LXP2.1 promoter, which is described as liver specific. LXP2.1 drives the expression of a codon-optimized, but CpG depleted, FIX-Padua gene. The vector was manufactured with triple-plasmid transfections of mammalian cells and purified to <20% empty particles.

In a single-center phase 1 study, BBM-H901 was administered intravenously at a dose of

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