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In vivo self-assembly of small RNAs in extracellular vesicles: a commentary on a new therapeutic paradigm

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Highlights:

- *In vivo* assembly links siRNA production with endogenous sEV release.
- Genetically encoded ligands provide a route to tissue-directed sEV delivery.
- Vesicle heterogeneity, dose definition, and repeat-dose safety remain key translational issues.

Abstract: Extracellular vesicles (EVs) can transport functional RNA, but the clinical development of EV-based RNA therapeutics has been constrained by the technical demands of *ex vivo* production and cargo loading. A recently reported strategy uses a plasmid to direct the formation of small interfering RNA (siRNA)-containing small EVs (sEVs) *in vivo*. Following administration, hepatocytes produce the therapeutic RNA and release it in association with endogenous sEVs, allowing systemic delivery to be achieved without isolating and reloading vesicles outside the body. This commentary examines the biological basis and potential therapeutic relevance of this approach. Particular attention is given to endogenous cargo loading, the use of genetically encoded targeting peptides to alter vesicle distribution, and the experimental issues that remain before clinical translation can be considered. These issues include control of vesicle composition, definition of the delivered dose, formulation-related toxicity, and the effects of repeated administration.

Keywords: extracellular vesicles; small RNA; *in vivo* self-assembly; siRNA delivery; RNA therapeutics

1. Introduction

Extracellular vesicles (EVs) have long been recognized as promising vehicles for RNA therapeutics due to their natural biocompatibility, low immunogenicity, and ability to cross biological barriers [1]. Yet, despite decades of research, clinical translation of EV-based RNA drugs remains limited [2]. The conventional paradigm—isolating EVs from cultured cells, loading therapeutic RNAs *ex vivo*, and reinjecting the engineered vesicles—is fraught with inefficiencies: low loading yields, batch-to-batch variability, high production costs, and potential damage to EV integrity during purification [3,4].

A conceptually different strategy has recently emerged from our group and others: *in vivo* self-assembly of small interfering RNAs (siRNAs) within endogenous small EVs (sEVs). By a single intravenous injection of a DNA plasmid encoding a therapeutic siRNA and a targeting peptide fused to an EV-anchoring protein, the liver is reprogrammed to continuously produce, package into sEVs, and distribute the siRNA systemically (Figure 1) [5]. This commentary focuses on why this paradigm constitutes a major advance for EV biology and therapeutics, and what questions it raises for the field.

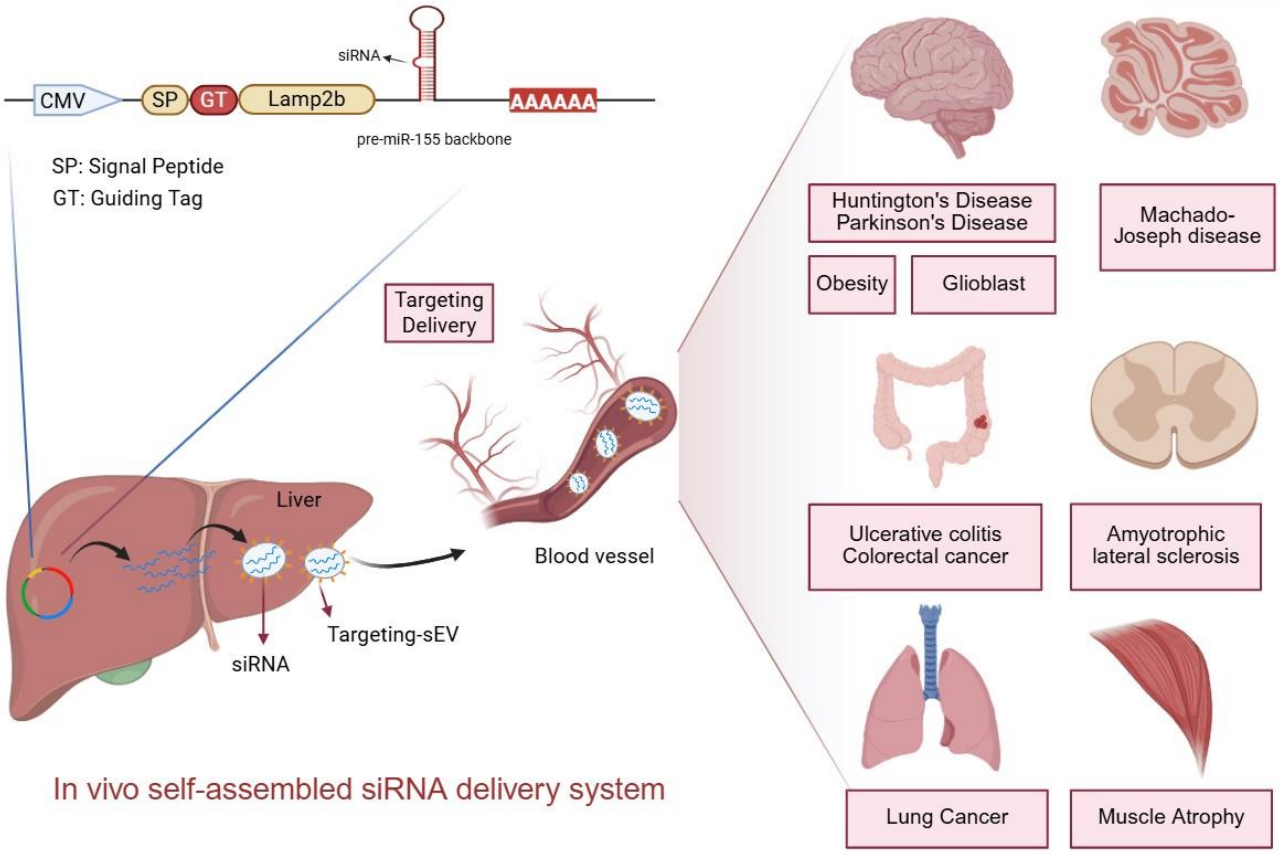

Figure 1. Schematic representation of the *in vivo* self-assembled siRNA delivery system and examples of disease models in which the platform has been evaluated (created with BioRender.com). The reported applications include neurological, metabolic, inflammatory, malignant, and muscular disorders. The disease examples shown in the figure are intended to illustrate the range of preclinical models examined and do not imply equivalent efficacy across indications.

2. The conceptual shift: from exogenous engineering to endogenous programming

The *in vivo* self-assembly system bypasses every major hurdle of traditional EV manufacturing. There is no cell culture, no purification, no external loading, and no synthetic carrier. The reported system uses the liver as the principal site for siRNA expression and sEV release [6–9]. This arrangement removes several operations that are required for conventional EV preparations, including cell expansion, vesicle isolation, post-production loading, and purification of the loaded product. It therefore changes the main engineering problem from improving the efficiency of loading an isolated vesicle to determining how endogenous vesicle biogenesis can be directed toward a defined therapeutic cargo.

Several biological observations from this system are particularly noteworthy for EV researchers.

(1) Endogenous loading confers superior bioactivity. The siRNA produced inside hepatocytes is associated with Argonaute 2 (AGO2) and other RNA-induced silencing complex (RISC) components before being packaged into sEVs [7,10]. The resulting complex is a pre-activated silencing machine that acts immediately upon entering target cells. This finding is consistent with the remarkable potency reported at plasma concentrations as low as 150 fM, which are lower than the concentrations commonly reported for many synthetic siRNA delivery systems [11]. It challenges the assumption that all siRNAs are functionally equivalent, highlighting that the "biogenesis history" of EV-encapsulated RNA determines its therapeutic efficacy. However, direct comparisons are difficult because EV-based and synthetic siRNA studies use different dosing units, formulations, administration routes, animal models, and pharmacodynamic endpoints

(2) EV tropism is programmable. By incorporating different targeting peptides (e.g., rabies virus glycoprotein (RVG) for the brain, muscle-specific peptide (MSP) for muscle) into the plasmid, the system redirects sEVs to distant organs, including across the blood-brain barrier [6,9]. This demonstrates that EV surface properties can be deliberately engineered at the source, without post-production modification. The modular design allows rapid swapping of targeting modules, making it a versatile platform for diverse indications.

(3) Simplified manufacturing and regulatory path. Since the final "product" is produced inside the patient, the need for centralized good manufacturing practice (GMP) manufacturing of EV drugs is eliminated. The plasmid itself is a well-characterized genetic material, and its transient expression (cleared within 24 hours) provides a built-in safety switch. This could significantly lower the cost and complexity of bringing RNA therapeutics to the clinic.

3. Key innovations of the *in vivo* self-assembly approach

This strategy introduces key innovations that distinguish it from previous EV-based therapies.

First, the strategy uses the liver's endogenous capacity for protein synthesis and sEV secretion. The resulting vesicles are formed under physiological conditions, which may help preserve membrane-associated proteins and reduce some of the stresses associated with vesicle isolation, storage, and post-production manipulation. Whether this advantage is maintained across different formulations, doses, and disease states remains to be determined experimentally.

Second, RNA synthesis and vesicle loading are linked in the same biological system. In conventional preparations, therapeutic siRNA is added to vesicles after vesicle formation, and the loading step may be inefficient. One cited study reported an encapsulation efficiency below 10% for a conventional approach [12]. In the *in vivo* system, newly transcribed siRNA is available to the endogenous sorting machinery as sEVs are formed. The extent to which this process represents active sorting, passive association, or a combination of both requires direct measurement.

Third, the transient expression of the plasmid may provide a degree of temporal control over production. The available report indicates that plasmid expression in the liver declines within approximately 24 hours. This observation is encouraging, but it should not be equated with a validated safety mechanism. Plasmid persistence, tissue-specific expression, the effect of repeated dosing, and the influence of the delivery formulation will determine whether production can be controlled predictably *in vivo*.

Fourth, tissue targeting is encoded at the level of the producer cell. A targeting peptide fused to the EV membrane protein Lamp2b can be incorporated into newly released sEVs, as illustrated by the use of RVG and MSP sequences in different experimental settings. This design avoids a separate chemical conjugation step and permits the targeting module to be changed by modifying the corresponding DNA sequence. The uniformity of peptide display and the possibility of incorporating more than one targeting ligand nevertheless require quantitative characterization at the single-vesicle level.

Fifth, the genetic construct can be adapted by changing the siRNA sequence or the targeting module. The platform has been examined in several disease models, including neurological, muscular, oncological, inflammatory, metabolic, and antibacterial applications [6,7,9,10,13]. These studies indicate that the design is adaptable, although each new cargo, target tissue, and disease context may require independent optimization of expression, vesicle composition, biodistribution, and dose. The evidence therefore supports platform versatility but does not by itself establish that performance will be equivalent across indications.

Finally, the inducible nature of the system may be useful for studying EV biology *in vivo*. A defined genetic input provides an experimental reference point for examining the timing of sEV release, tissue distribution, and cargo uptake under physiological or pathological conditions. Such experiments could complement *ex vivo* studies, particularly when the objective is to resolve how EV-mediated communication changes between organs. This application will depend on the ability to distinguish newly induced vesicles from the endogenous vesicle pool and to measure cargo delivery rather than particle distribution alone.

Taken together, these characteristics define a different way of producing therapeutic EVs. The approach may reduce several manufacturing steps and provides genetic control over cargo and targeting. Its broader value will depend on whether vesicle composition, dose, biodistribution, and biological activity can be measured and controlled with sufficient precision for preclinical and clinical use.

4. Critical research gaps and translational considerations

Although the platform has shown therapeutic effects in preclinical models, its suitability as a clinically reproducible EV production strategy remains unestablished. sEV yield and composition may vary with plasmid delivery, hepatic condition, expression duration, and other host-related factors. The administration system also requires further assessment, particularly with respect to innate immune activation, inflammation, complement activity, biodistribution, liver injury, coagulation, immunogenicity, and repeat-dose tolerability.

It is also unclear whether individual sEVs consistently carry both the targeting ligand and therapeutic siRNA. Co-expression of Lamp2b and siRNA in producer cells does not demonstrate their co-occurrence within the same vesicle. This heterogeneity could affect tissue targeting and complicate dose estimation. Single-vesicle or rigorously fractionated analyses should determine particle number, surface-ligand display, cargo abundance, and the relative contributions of active sorting and passive loading. These data may guide the development of more effective sorting elements and potency-based quality criteria.

The reported plasma concentration of approximately 150 fM is encouraging but should not be regarded as a general potency benchmark. Differences in administration route, formulation, animal species, target tissue, and reporting units limit direct comparison across studies; independent replication under standardized conditions is still needed [5]. Likewise, transient plasmid expression may offer some control over production, but its duration and safety after repeated dosing require evaluation in larger-animal models.

5. Conclusions

In vivo assembly of siRNA-containing sEVs offers an alternative to conventional EV isolation and *ex vivo* loading. By using hepatic vesicle biogenesis, the system allows the siRNA sequence and targeting ligand to be encoded in a single construct and has been tested in several preclinical models. Further studies are needed to define vesicle heterogeneity, formulation safety, dose, biodistribution, and the effects of repeated administration. Clinical translation will require measurements that link vesicle composition with tissue exposure and pharmacological activity, rather than relying solely on total particle numbers or plasma RNA levels.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. Specifically, the authors used Manus for language polishing in limited sections. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Conceptualization, X.C. and X.Y.; writing—original draft preparation, A.A.; writing—review and editing, A.A. and X.Y.; funding acquisition X.C. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

Xi Chen holds the position of Executive Editor for *ExRNA* and has not peer reviewed or made any editorial decisions for this paper. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors disclose that some cited studies were conducted by members of the authors' research group; the commentary is intended to provide a balanced assessment of the platform, including its current limitations.

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