RNA Nanotechnology: New Hope for Drug Delivery on the Nanoscale

A novel nanoparticle, the pRNA nanoparticle, has demonstrated the desired pharmacological properties as well as manufacturability, suggesting that it could represent a powerful platform for the efficient delivery of therapeutics to treat cancer and inflammatory diseases.

Nanotechnology has been shown to be a promising approach for delivering drugs and diagnostics. A newly developed RNA nanotechnology has many unique and preferred pharmacological and other properties that are able to overcome problems faced by many of today’s nanotechnologies and are key to the development of a successful pharmaceutical product. The RNA nanoplatform may represent a new generation of nanodelivery systems appearing on the horizon for pharmaceutical applications, including the delivery of RNAi and small molecules.

THE NANOPARTICLE IN DRUG DELIVERY

The nanoparticle has been increasingly recognised as a preferred drug delivery system (1). A particulate structure on the nanoscale (10–100 nm) has several favourable pharmacological properties over free drug delivery:

- It improves the solubility of otherwise poorly soluble hydrophobic agents, without the need for an undesired excipient to facilitate administration
- It improves pharmacokinetic (PK) behaviour (for example, extended half-life or $T_{1/2}$) because of its large size (avoiding kidney clearance), provides protection from degradation and has a sustained release mechanism
- It improves biodistribution by reducing extravasation (smaller volume of distribution, $V_d$) and thus exposure to normal tissue and potential damage. It accumulates and concentrates in certain disease tissues, for example, tumours and inflammation sites, as a result of enhanced permeability and retention (EPR), thereby increasing bioavailability at disease sites and thus enhancing specificity
- A single particle can be functionalised with multiple functions: ligands targeting disease tissues/cells and therapeutic payloads of different mechanisms of action (MOA); and diagnostic agents for diagnosis or theranostics
- The nanoparticle can deliver macromolecular drugs (proteins, nucleic acids and so on) that would otherwise be too difficult to deliver

LIMITATIONS OF CURRENT ANTICANCER THERAPY

Chemotherapy is the main treatment option for metastatic cancers. However, it only provides certain benefits for some cancers, while having little clinical benefit for many others, such as advanced staged pancreatic, ovarian and lung cancers. Its failure can be attributed to a lack of specificity (narrow therapeutic window, see Figure 1) and low bioavailability, as well as the development of drug resistance. Overcoming these problems has proven to be challenging, and past efforts have yielded only limited improvement:

- Few agents have been found to be truly cancer-specific
- Few truly cancer-specific receptors can be exploited for specific targeting
- Efforts to increase drug bioavailability at tumour sites are hindered by the simultaneously increased distribution into normal tissue, leading to dose-limiting toxicity (DLT)
- Optimal combination treatments with synergistic efficacy but without overlapping toxicity help fight drug resistance and increase efficacy, but identifying an optimal combination has proven to be difficult because of the distinct PK profiles of different agents

Nanotechnology, however, offers a unique opportunity to overcome many of these pitfalls and to improve cancer treatment (see Figure 1).

CHALLENGES IN DEVELOPING NANODELIVERY

Many factors influence the utility and effectiveness of a nanodelivery system. They can be divided into two categories: factors related to manufacturing and quality...
control (CMC, see 1-3 below); and those related to pharmacological attributes (4-5 below):

1. Stability affects manufacturing, storage and potency
2. Controllable stoichiometry and self-assembly allow for simple and reproducible manufacturing
3. Modular design leads to robust functionalisation
4. Biocompatibility, metabolic stability and particle size directly affect pharmacological properties including PK, tissue distribution, cellular uptake and toxicity
5. Engineered targeting mechanisms can specifically guide delivery to the diseased tissues/cells/subcellular locations or pathogens

However, none of today's nanotechnologies has all of these features, and many only have a few. For example, common liposome- and polymer-based nanoparticles all suffer from poor biocompatibility, being large (>100 nm), non-uniform and unstable, resulting in undesired biodistribution with predominant accumulation in the liver and spleen, and causing DLT. They also usually require a complex and inconsistent manufacturing process because of the lack of controlled stoichiometry. These challenges are similarly faced by today's RNAi nanodelivery systems.

RNA NANOTECHNOLOGY

RNA nanotechnology is an emerging technology that promises a bright future because it possesses several enabling features that make for an attractive delivery system (2):

◆ RNA molecules can possess various functions that can directly be exploited as ligands (aptamers) and therapeutic payloads (for example, RNAi, ribozymes)
◆ As a nanomaterial from nature, it is biocompatible, non-immunogenic and biodegradable, promising a safe ‘green’ nanotechnology with no DLT
◆ It can form diverse, defined and stable nanostructures (secondary-2˚, tertiary-3˚, quaternary-4˚) resulting from strong base pairing (ΔG of UC/AG = -2.4 kcal/mol) and other interactions (2). As an informational macromolecule, its structures and modularity are hierarchically manifested on the chemical, structural and supramolecular levels, and coded in its sequence. RNA molecules can be fabricated for different sizes/structures for modular design and can be assembly programmed using fully addressable building blocks (a ‘bottom-up’ approach). In other words, the RNA nanoparticle can be rationally constructed using 3/4˚ structures created by sequence (1˚) manipulation (3,4)

◆ The availability of total synthesis and stoichiometrical modifications affords industrial-scale manufacturing, as well as robust functionalisations

Although achievable in theory, no rationally engineered nanoparticle has been designed and developed for pharmaceutical applications so far; this is due to the extreme variability of RNA structures and the lack of effective/reliable tools. Nature has fortunately provided us with an evolutionally selected RNA nanoscaffold with the features that we desire.

pRNA NANOTECHNOLOGY FOR DRUG DELIVERY

Bacterial phage φ29 packaging RNA (pRNA) is a naturally occurring molecule with the right features for an RNA nanoscaffold (5). The 117-nucleotide pRNA molecule constitutes one of the six pRNA subunits (monomers) of the packaging motor and is responsible for packaging virion DNA into procapsids. Research by Dr Peixuan Guo and colleagues at the University of Cincinnati (OH, US) has revealed that pRNA folds tightly into a stable 2˚/3˚ nanostructure of 11 nm, and can multimerise to form dimers, trimers, tetramers, and hexamers of different sizes (see Figure 2, page 76). While the core scaffold tolerates few changes, certain positions have been identified to accommodate modifications for stabilisation/functionnalisation. For instance, the 5/3˚ duplex in pRNA accommodates sequence changes and enables pRNA to be readily engineered as an RNAi trigger termed ‘pRNA interference’ or ‘pRNAi’ (6). pRNAi has been shown to be efficiently processed by Dicer and to silence the intended targets.

Figure 1: Therapeutic window and dose-limiting toxicity (DLT) of conventional therapies compared with nanotechnologies
Kylin has licensed pRNA nanotechnology and aims to develop it into a nanodelivery platform for therapeutics. A total synthesis strategy has been developed by Kylin scientists to produce the building blocks that can self-assemble from the bottom up into a functional pRNA monomeric nanoparticle (7) (see Figure 3), that can further multimerise to form larger particles. This entire process is highly scalable, consistent and suitable for pharmaceutical-grade manufacturing. The high modularity accommodates broad applications that would be difficult to achieve with other nanotechnologies. The nanoparticle is highly stable (thermo-dissociation, $T_{m} \approx 80^\circ$C) – a property that not only facilitates crucial manufacturing and functionalisation but also benefits development as a stable drug product.

At Kylin, we have recently conducted a comprehensive pharmacological evaluation of the engineered pRNA nanoparticle using both in vitro and in vivo experimental models. We have demonstrated that the particle is metabolically stable in biological milieu, and has a superior PK profile characterised by an initial rapid distribution upon systemic administration and a subsequent slower elimination with dose-proportional systemic exposure. The nanoparticle of 11 nm has a half-life ($T_{1/2}$) of around 7-13 hours and slow clearance ($Cl < 0.1$ L/kg/hr); this is likely due to the fact that it is metabolically stable, is larger than the kidney filtration threshold and is sufficiently small and hydrophilic to evade clearance by the reticuloendothelial system (RES) (7,8). The pRNA nanoparticle also shows modest extravasation ($V_d \approx 0.85-1.3$ L/kg), preventing over-exposure to normal tissues.

The pRNA nanoparticle did not induce an interferon response in vitro, nor did it induce cytokine production in vivo upon systemic exposure (7). Repeat IV injection did not cause any toxicity (seven days and up to 30 mg/kg < no observable effects limit, NOEL) (7). This non-toxic nature distinguishes it from other existing nanotechnologies that are plagued with DLT.

At Kylin, we have now developed a specific chemistry that allows robust functionalisation of the pRNA nanoparticle with therapeutic payloads – including RNAi and small molecules – and which can further be released once they enter cells for the treatment of human diseases. Kylin's first pRNAi nanoparticle candidate silences the survivin oncogene in cancer cells, sensitising them to apoptosis. Since survivin is a validated cancer target for many different types of human cancer, including ovarian cancer, and since many different cancers over-express the folate receptor – particularly ovarian cancer (>90 per cent) – we anticipate that this lead

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![Figure 2: The size and structure of pRNA nanoparticles. Top left: monomer; Top right: trimer; and Bottom, left and right: AFM images](image)

![Figure 3: Simple, consistent and cost-effective manufacture of functional pRNA nanoparticle](image)
pRNAi nanoparticle will have potential as an effective RNAi therapeutic approach in the treatment of ovarian cancer.

In addition, Kylin is also developing new cancer therapies by designing nanoparticles delivering: a) a highly potent cytotoxic agent that was shown to be too toxic in previous clinical trials; b) agents with poor drug properties (poor solubility, PK and tissue distribution); and c) the combination of agents with precisely defined ratios and with the known synergistic efficacy but without overlapping toxicity. Kylin predicts that the pRNA-nanodelivered cytotoxic agents will increase drug bioavailability in the targeted tumour cells and reduce distribution/toxicity in normal tissue, as compared with the same free drugs, thus improving the efficacy and safety of the drug treatment.

PERSPECTIVES

RNA nanotechnology has shown great potential for drug delivery compared with other nanotechnologies. It is gaining recognition as a new generation of nanotechnology for medical applications, due to its capacity for modular design and self-assembly, and its total biocompatibility and safety profile. As the first RNA nanoparticle that has been comprehensively characterised – particularly in vivo using animal models – the pRNA nanoparticle has demonstrated adequate pharmacological properties and manufacturability, suggesting that it could be developed as an enabling drug delivery platform for the delivery of different therapeutic agents – including small molecules and RNAi – with great potential to advance the treatment of human diseases – notably cancers and inflammatory diseases – in the near future.

References