

Editorial

Nanocarriers for AKT siRNA Based Gene Therapy

Dubey P¹ and Gopinath P^{1,2*}¹Centre for Nanotechnology, Indian Institute of Technology Roorkee, India²Department of Biotechnology, Indian Institute of Technology Roorkee, India***Corresponding author:** Gopinath P, Department of Biotechnology, Nanobiotechnology Laboratory, Indian Institute of Technology, Roorkee, Uttarakhand-247667, India**Received:** April 27, 2016; **Accepted:** May 03, 2016;**Published:** May 05, 2016

Editorial

In the past few decades significant attention has drawn for gene silencing strategies based on RNA interference (RNAi)-mediated knock down of oncogene targeted cancer therapy [1]. RNAi refers to a method, where specific protein suppression could be achieved by delivery of double stranded short (20-25bp) interfering RNA (siRNA) [2]. However, the successful RNAi mediated gene therapy depends upon effective intracellular delivery of siRNA either as preformed siRNA or in conjugation with expressing plasmid vector and the efficient knock-down of oncogene transcripts [3].

Among various oncogenic targets, AKT/Protein Kinase B (PKB) remains the central player in cell signaling pathways, altering cell survival and death [4]. Its activation leads to apoptotic resistance in cells, support cell survival, growth, and migration, energy metabolism and angiogenesis. It is evident from various studies that AKT perturbations plays an important part in tumorigenesis, [5] based on constitutive and increased expression of various AKT isoforms in diverse cancers, the inactivation of antagonists such as Phosphatase Tensin Homolog (PTEN), or mRNA over expression [6,7]. Its anti-apoptotic action accounts for cell transforming ability and drug resistance in cancer cells against various chemotherapeutic agents [8]. Hence, AKT appears to play a pivotal role in the growth and tumor cells survival. Activation of Phosphatidylinositol 3-Kinase (PI3K)/AKT due to genetic alteration leads to chemotherapeutic insensitivity in diverse cancer preclinical and clinical trials [7,9]. Its dysregulation showed profound effect on the sensitivity of doxorubicin and 4-hydroxyl tamoxifen toward breast cancer chemotherapeutics [10]. Furthermore, characteristic AKT activation has been observed in various human tumor malignancies thus resulting in unfortunate predictive results [11,12]. Three AKT isoforms: AKT1/PKB α , AKT2/PKB β , and AKT3/PKB γ namely found in mammalian tissues, among which AKT1 and AKT2 has been found to be ubiquitously expressed in all type tissues observed and upregulated in various transformed tissues [5]. All these isoforms share amino acid homology closely and gets activated by PI3K-dependent pathway [2].

Consequently, AKT regulation possesses tremendous therapeutic attention and can be accomplished by successful delivering of AKT-siRNA. But for successful AKT-siRNA delivery, capable and safe nanocarrier is utmost necessity, and has appeared as major hurdle in

siRNA based therapeutics. Though the RNAi is naturally occurring process in cells which provide all necessary components including the formation of RNA-Induced Silencing Complex (RISC) induces dicer endonuclease mediated cleavage of mRNA as showed in Figure 1a [13]. However the delivery of therapeutic siRNA is essential for induction of RNAi. Thus difficulties in the systemic delivery of siRNA to targeted tissues due to poor intracellular uptake, immunogenic response and limited blood stability hinders the siRNA therapeutics [14]. Additionally, delivery of siRNA to the target tumor site has been another hurdle in gene therapy. Furthermore, the bare siRNAs do not able to easily cross the cell membrane due to their negative charge and size [15].

Therefore in order to augment the intrinsic therapeutic potency and delivery of siRNA, various nanocarrier-based targeted gene therapies have explored (Figure 1b) [16]. Among various nanocarriers, viral vectors have been explored primarily [17]. Despite providing high transfection efficiency, their applications were limited by immunotoxicity, chances of occurrence of mutagenicity and tumorigenesis. Considering the safety concern the non-viral vectors have been investigated such as various natural and synthetic polymers, cationic lipids and metal nanoparticles etc., [18]. Among which the cationic liposomes have exhibit hopeful results as delivery

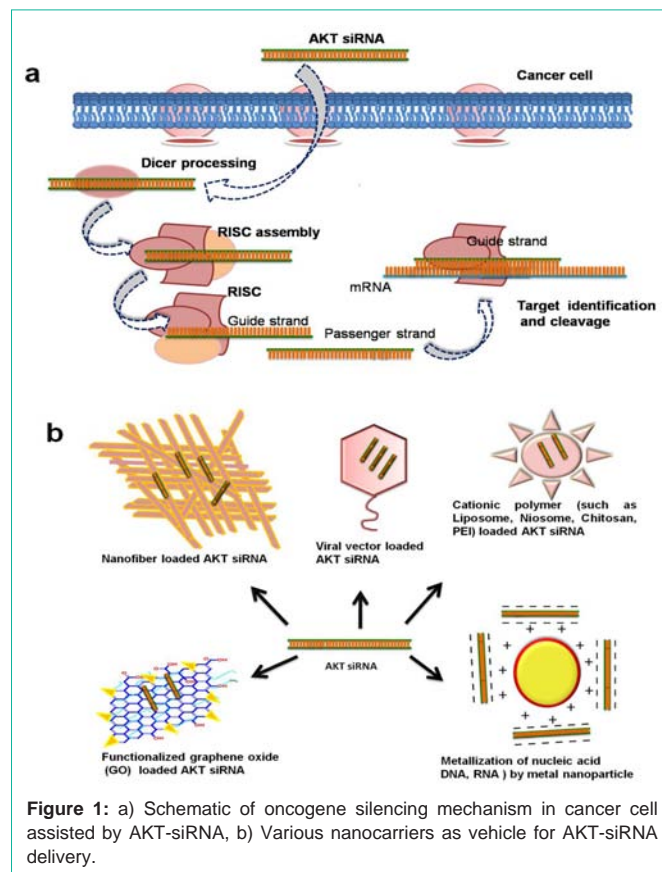


Figure 1: a) Schematic of oncogene silencing mechanism in cancer cell assisted by AKT-siRNA, b) Various nanocarriers as vehicle for AKT-siRNA delivery.

vector by protecting the siRNA against nucleases and evading the endosomal degradation [19,20]. Nevertheless, the use of liposomes was limited based on their reduced stability in physiological medium, short circulation life, and lack of controlled release system. Recently, a new class of liposomes known as niosomes is evolved as an alternate with enhanced rigidity, stability, biocompatibility, and high dispersive nature that avoid the blockage of vessels [21].

Among cationic natural polymers, the chitosan based nanocarrier has been successfully applied for *in vitro* and *in vivo* gene delivery [22-25]. It is a mucoadhesive, biodegradable and biocompatible, cationic polysaccharide, however, its low transfection efficiency based on its poor buffering ability for intracellular endosomal escape remained the challenge. Studies have been done to further improve its transfection efficiency by grafting it with synthetic cationic polymer, the Polyethylenimine (PEI) (CHI-g-PEI) [26] which showed better cell viability *in vitro*, and *in vivo* with enhanced transfection efficiency on aerosol delivery [2,26-28].

Therefore among synthetic polycationic polymers used for siRNA delivery, PEI take an important place based on its comparatively high gene transfection efficacy [29,30]. It is a water-soluble polymer with protonable amino groups, provides high cationic charge density at physiological pH. Various modifications of PEI with several ligands or coupling agents as cell-specific moieties are promising approaches to enhance the specificity, biocompatibility, and transfection. In physiological conditions, PEI remains protonated which allows it to form electrostatic complexes with nucleic acid molecules called as 'polyplexes' or nanoplexes which offers the shield for siRNA from nucleolytic enzyme degradation, promote efficient uptake via endocytosis and intracellular release through the phenomena called as 'proton sponge effect' [27]. These polyplexes enter the cells via caveolae- or clathrin mediated pathways, where with the former route leads to efficient transfection. A study showed biodegradable nano-polymeric systems based on poly (ester amine) carrier based on Polycaprolactone (PCL) and PEI which showed impressive *in vitro* and *in vivo* gene delivery [30].

Recently, Graphene Oxide (GO) has been came in limelight based on its exceptional properties which leads to extensive investigation of GO for various applications including the drug delivery, biosensors, bioimaging, and gene therapy. The first study of GO as gene delivery vehicle was done by Liu and co-workers in combination with PEI, which was then tagged with EGFP plasmid DNA (pDNA) for intercellular gene transfection in HeLa cells. In another study Zhang and co-workers prepared PEI-grafted GO (GO-PEI) through covalent modification as an excellent nanocarrier for delivery of siRNA and drugs *in vitro* [31]. Recently the GO-PEI-PEG functionalized nanocarrier has been explored with an excellent physiological stability and solubility with low toxicity for delivering siRNA, CpG, STAT3, VEGF etc into cells [32-34].

Nanofibers have been widely explored for numerous applications, including antibacterial, cancer cell and tissue engineering field [35,36]. Recently the electrospun nanofibers were explored for their gene therapy potential [37]. As a template for nucleic acid delivery, nanofibers offers various advantageous features including the ease of production, their ECM-mimic behavior, feasibility of providing various properties by modifications, and their large surface area. It

exhibits a great ability to control the release kinetics of gene vectors and enhance gene delivery efficiency [38,39].

Recently the concept of DNA metallization has been expanded for gene delivery. A study reported facile synthesis of pDNA-templated silver nanoparticles (Ag NP), which could serve as a platform for effective gene delivery [40,41]. Compared to conventional nanocarriers, the metalized-pDNA offers numerous advantages, including providing appropriate size and surface charge, facile synthesis and minimal cytotoxicity, thus biocompatible nanomaterials for efficient gene delivery.

Though many studies are done for exploration of gene delivery nanocarriers, however the clinical translation of these nanocarriers is the major hurdle due to the non-specificity, cytotoxicity, biocompatibility and stability in physiological milieu emerged as critical bottleneck for siRNA therapeutics. Hence for successful siRNA delivery, safe and efficient gene delivery system is imperative.

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