

# Intrinsic Bio-Signature of Gene Delivery Nanocarriers May Impair Gene Therapy Goals

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## SUMMARY

Non-viral lipid/polymeric vectors have widely been used as nanocarriers (NCs) for gene delivery. They possess large surface area to volume ratio and are able to interact with biomolecules through functional moieties, resulting in inadvertent biological impacts, in particular at genomic level. Thus, their genomic bio-signature needs to be investigated prior to use *in vivo*. Using high-throughput microarray and qPCR gene expression profiling techniques, we have reported the genomic impacts of lipid/polymeric NCs. Given the fact that the ultimate objectives of gene therapy may inevitably be impaired by nonspecific intrinsic genomic impacts of these NCs, here, we highlight their nonspecific genomic bio-signature. We envision that better understanding on the genotoxicity of gene delivery NCs, as guiding premise, will help us to develop much safer NCs and also to accelerate their translation into clinical use and to provide pivotal information on safety liabilities early in discovery and developments process prior to its inevitable consequences *in vivo*.

The principle of gene therapy possesses undeniable therapeutic advantages over the conventional therapeutic modalities that are basically dependent upon exploitation of small molecules or biological pharmaceuticals. These advantages are: a) specific or selective treatment of diseased cells/tissue, b) minimal adverse consequences, c) correction of the genetic cause of a disease, and d) long-term treatment after single application.<sup>1,2</sup> To silence/suppress a target gene or to correct a genetic defect, the gene-based therapeutics such as antisense oligonucleotides (ASODNs), plasmid DNA, ribozymes, DNazymes and siRNAs need to be shuttled to the target site.<sup>3-6</sup> However, despite implementation of various strategies for delivery of gene medicines (e.g., viral and non-viral vectors as well as physical methods such as microinjection, electroporation, gene gun, ultrasound and hydrodynamic delivery),<sup>7-11</sup> safe gene transfer into various target cells still faces major obstacles including: poor delivery efficiency, cellular toxicity, immunogenicity, oncogenicity, short-term transgenic expression and poor expression levels.<sup>1,12</sup> For systemic gene delivery, viral and non-viral vectors have been widely used. Ideally, in either *ex vivo* or *in vivo* approach for gene therapy, only the therapy-intended gene expression changes should occur, nevertheless this is not always the case. Of the most commonly used gene delivery systems, viral

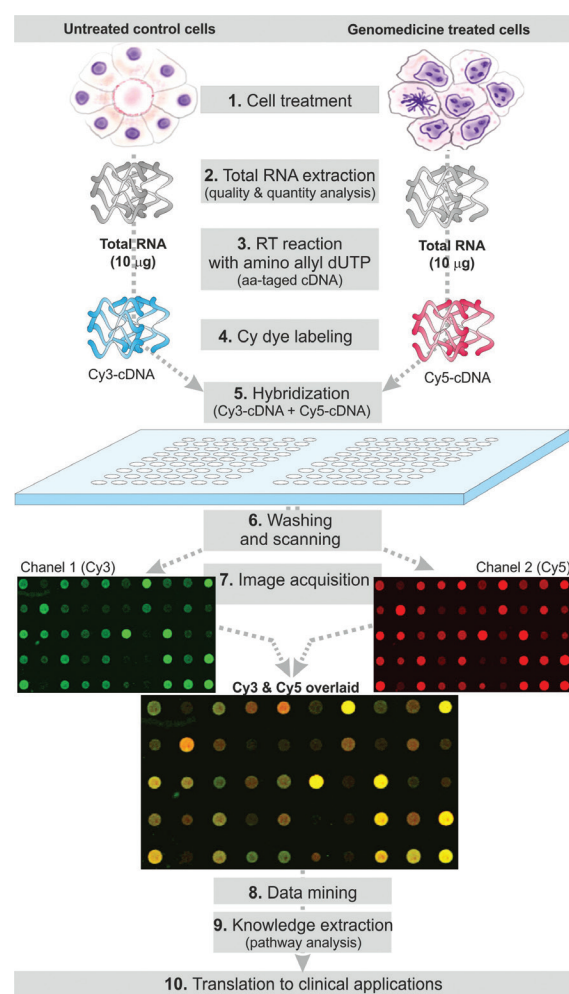
vectors (e.g., retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses) are known to be efficient delivery systems for nucleic acids while they can induce immunogenic responses,<sup>13-15</sup> with serious consequences as seen in the disastrous death of Jesse Gelsinger, who suffered from ornithine transcarbamylase deficiency and went under gene therapy using adenoviral vector carrying the corrected gene. These problems decelerated human gene therapy modalities and highlighted use of safer non-viral vectors. It should be also noted that *in vitro* gene therapy of cells with ASODNs for a short period of time (3-6 h) were shown to induce desired effects, while *in vivo* gene therapy demands repeated administration through multiple injections for prolonged exposure of target cells to ASODNs.<sup>3</sup> All these issues highlight the necessary use of safe biocompatible lipid/polymeric carries for gene delivery even though the transfection efficiency of viral vectors is greater than that of the non-viral vectors. Hence, several non-viral gene delivery nanosystems such as cationic polymer- or lipid-based formulations have been developed for nucleic acid delivery.<sup>16-18</sup> These cationic nanostructures can readily condense DNA into complexes and form polyplexes/lipoplexes to be used for *ex vivo* and *in vivo* gene therapy.<sup>17,19</sup> So far, many advanced nanomaterials (NMs) have been used as non-viral gene delivery system. While *in vitro* and *in vivo* preclinical

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applications of advanced NMs and NCs as molecular therapy continue to increase, their molecular signature (the so-called nanotoxicology) should effusively be investigated prior to their translation into human subjects. To disclose the nanotoxicological impacts of NMs and NCs, implementation of high throughput molecular profiling technologies appears to be inescapable. In fact, molecular impacts of these NMs may delineate the molecular toxicity mechanisms and pathways involved in repair, survival and/or cell death.<sup>20</sup> To understand such molecular mechanism(s) of NMs/NCs in target cells, we need to look at the molecular pattern, in particular gene expression changes pattern using high throughput gene expression profiling techniques. Various techniques have been capitalized for high throughput gene expression

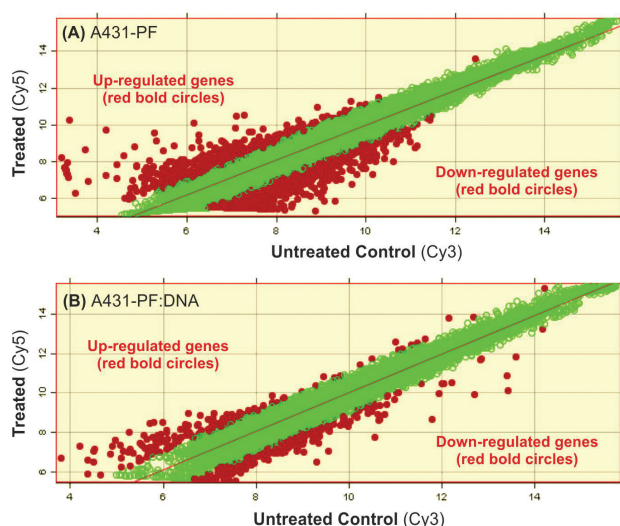
profiling including: DNA microarray, serial analysis of gene expression (SAGE), single promoter-reporter biosensors sensitive to DNA damage in mammalian cells<sup>21</sup> and next generation RNA sequencing.<sup>22</sup> Of these approaches, DNA microarray technology, which has been established almost two decades ago,<sup>23</sup> appears to be an effective method for large-scale global gene expression analysis,<sup>24</sup> leading to emergence of genomic toxicity assessments, so-called as “*Toxicogenomics*”.<sup>25-29</sup> We have utilized non-viral vectors for gene delivery,<sup>30-35</sup> and widely used DNA microarray technology to determine intrinsic gene expression alteration potential of NMs/NCs used as gene delivery system.<sup>26-29, 33-38</sup> Fig. 1 represents schematic illustration of DNA microarray steps.

Depending on NCs surface charge and functional moieties,



**Fig. 1.** Schematic representation of DNA microarray procedure. The cultivated cells, at 40-50% confluency, are treated with a designated non-viral vector/genomedicine (1). The total RNA is extracted from the treated (T) and untreated (UT) cells and qualitatively and quantitatively analyzed (2). Reverse transcription (RT) is performed using total RNA (10 µg) and designated ratio of aminoallyl-dUTP:dTTP and RT products (cDNA) are purified (3). The T and UT cDNAs are labeled with cyanine dyes, i.e. Cy3 and Cy5, and the Cy3- and Cy4-labeled cDNAs are purified and quantitatively analyzed (4). The labeled cDNAs are hybridized on a glass slide array overnight (5). The slide arrays are washed and scanned for both Cy3 and Cy5 channels (6). Image acquisition is performed— Cy3 (green), Cy5 (red) and superimposed images are shown as examples (7). Data mining (8) and knowledge extraction (e.g., gene ontology, pathway analysis) are accomplished using designated software (9). The resultant data are translated into clinical applications (10).

they can intrinsically induce profound biological effects in target cells. It is not clearly understood how the internalized nanoparticles (NPs) can elicit inadvertent cellular impacts such as mutagenicity, inflammatory response and DNA damage.<sup>39</sup> Hence, it is important to shed some lights on the genocompatibility and toxicogenomics of NCs. Surprisingly, little is known upon intrinsic bio-signature of gene delivery NCs. Based on our microarray screening findings, most of non-viral gene delivery NCs appear to interfere with the main objectives of such therapy by masking/stimulating nonspecific genes due to their own genomic impacts.<sup>26-28,37,38,40,41</sup> Non-viral polycations are principally able to condense, enhance the delivery and improve the biological end-point of nucleic acids; however, they often exert cytotoxicity which is dependent on delivery system/target cells.<sup>42</sup> Thus, both transfection efficiency evaluation and safety assessment are essential for gene transfer with these gene therapy vectors.<sup>43-45</sup> Fig. 2 represents the scatter plots of gene expression changes induced by cationic polymer (Polyfect™) alone or as polyplex in A431 cells. As shown in Fig. 2, a large number



**Fig. 2** Scatter plots of gene expression changes induced by cationic polymer alone or as polyplex in A431 cells. Panels A and B respectively represent the genomic impact of Polyfect™ (PF) and its complex with scrambled DNA. PF:DNA Polyplex induced significantly lower gene expression. Each circle conveys a designated gene. Red circles represent up-regulated or down-regulated genes, and green circles show unchanged genes.

of various genes can be upregulated by cationic NCs, while complexed with DNA as polyplex the alteration in gene expressions was reduced perhaps because of reduction in surface charge of polymeric carrier. A number of factors may affect the efficacy and safety of non-viral vector-mediated gene transfer, in particular their structural properties and type of target cells and tissue.<sup>46-48</sup> We have tested various polymeric and lipidic NCs and found that the gene expression changes correlate

with surface charge and molecular structure and degree of biodegradation of NCs. For example poly (lactic-co-glycolic acid) (PLGA), as a biodegradable polymer, was found to be safer than cationic non-biodegradable polymers.<sup>49</sup> Further, the branched molecular structures appear to induce greater genomic impacts than the linear ones as previously reported for the cytotoxic impacts of branched and linear polyethylenimine nanostructures in A431 cells.<sup>40</sup> Surprisingly, despite these facts, little attention has been paid for validation of gene therapy strategies through proof-of-concept studies solely for the influence of carriers of genomedicines. Given the fact that various target cells may display different responses, the transfection efficiency and safety of vectors should also be optimized carefully based on types of target cells and target organs.<sup>50</sup> Once target cells were transfected, specific attention should be given to the genotoxicity potentials of gene-based medicines. In fact, the definitions and purposes of gene therapy have been sharply focused, but the actual implementation of the task has not kept pace due to many unanticipated roadblocks.<sup>51</sup>

In short, NCs used for delivery of nucleic acids are able to inherently induce inadvertent alterations in gene expression. Therefore, since we literally look at the impact of active agents but not the vehicles, we need to be cautious upon inevitable bio-signature (molecular impacts) of NCs that may interfere with the main goal of gene therapy modality.

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#### Ethical issues

The authors declare no ethical issues.

#### Competing interests

The authors declare no conflict of interests.

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