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Nanomaterials for Cancer Precision Medicine

Yilong Wang, Shuyang Sun, Zhiyuan Zhang, and Donglu Shi**

Medical science has recently advanced to the point where diagnosis and therapeutics can be carried out with high precision, even at the molecular level. A new field of "precision medicine" has consequently emerged with specific clinical implications and challenges that can be well-addressed by newly developed nanomaterials. Here, a nanoscience approach to precision medicine is provided, with a focus on cancer therapy, based on a new concept of "molecularly-defined cancers." "Next-generation sequencing" is introduced to identify the oncogene that is responsible for a class of cancers. This new approach is fundamentally different from all conventional cancer therapies that rely on diagnosis of the anatomic origins where the tumors are found. To treat cancers at molecular level, a recently developed "microRNA replacement therapy" is applied, utilizing nanocarriers, in order to regulate the driver oncogene, which is the core of cancer precision therapeutics. Furthermore, the outcome of the nanomediated oncogenic regulation has to be accurately assessed by the genetically characterized, patient-derived xenograft models. Cancer therapy in this fashion is a quintessential example of precision medicine, presenting many challenges to the materials communities with new issues in structural design, surface functionalization, gene/drug storage and delivery, cell targeting, and medical imaging.

1. Introduction to Precision Medicine

Precision medicine, as defined by National Institutes of Health (NIH), is a medical strategy with individually customized healthcare. Fundamentally different from the conventional methodology, the precision theranostic procedures are designed, considered, and tailored to the individuals at the genetic level for optimum medical intervention.[1,2] The new definitions of medical theranostics may consequently facilitate a major overhaul of healthcare policies and procedures. Recent studies on gene expression profiling and genomic sequencing have revealed remarkable molecular heterogeneity within the disease. This tumor complexity requires the genetically characterized patient-derived xenografts (PDX) for more accurate assessment of drug efficacy. Previous research has shown tumor heterogeneity resulting from different genomic landscapes, environments, and tissue ecosystem, which evolve from iterative processes of clonal expansion, genetic diversification, and clonal selection within the adaptive tissue ecosystem.[3] This inherent tumor characteristic is highly individualistic, therefore demanding precision, personalized, and specific diagnosis and treatment at all levels, particularly at genetic level.[4]

The foundation of precision medicine in tumor theranostics is identification of the oncogene that is responsible for a class of common cancers regardless of anatomical sites.[1,4] These driver genes can now be identified by advanced genome sequencing techniques.[5] Integrating genomic information with clinical treatments, it is possible to classify cancers based upon more relevant tumor molecular characteristics rather than anatomic and histological criteria.[1] In this fashion, cancers are more precisely defined and clinically classified by their signature oncogenes for precision medical treatment of individual patient. With the driver gene identified from each patient, the tumor can well be suppressed via regulating its target gene by systemic delivery of noncoding RNAs including "microRNA replacement therapy," an emerging strategy that is therapeutically precise at the genetic level in juxtaposition with conventional medical intervention.

The limited tumor targeting ability and poor cellular penetration of noncoding RNAs remains, however, a great challenge that necessitates a particular delivery system for gene therapy. Viral vectors have been considered as effective delivery systems, but their systemic toxicity and immunogenicity post major concerns in clinical settings.[6,7] Nonviral vectors, constructed with nanomaterials, have, therefore, become the promising and popular delivery systems in biomedical applications.[8] The current critical issues in gene or drug delivery deal with the difficulties in facile and scalable synthesis, inefficient RNA payload, biological barriers to gene/drug delivery, low cellular uptake, lysosomal escape, and systemic toxicity.[8] To address these

Dr. Y. Wang, Prof. D. Shi
The Institute for Translational Nanomedicine
Shanghai East Hospital
the Institute for Biomedical Engineering & Nano Science
Tongji University School of Medicine
Shanghai 200092, P. R. China
E-mail: donglu.shi@uc.edu
Dr. S. Sun, Prof. Z. Zhang
Department of Oral and Maxillofacial-Head Neck Oncology
Ninth People's Hospital
Shanghai Jiao Tong University
School of Medicine
639 Zhizaoju Road, Shanghai 200011, P. R. China
E-mail: zhzhy@sjtu.edu.cn
Prof. D. Shi
The Materials Science and Engineering Program
College of Engineering and Applied Science
2901 Woodside Drive
Cincinnati
University of Cincinnati
Cincinnati, OH 45221, USA

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challenges, a variety of nanocarriers have been designed and developed, such as cationic lipids, polymeric micelles, polycation polymer-based carriers, and inorganic nanoparticles, with specific sizes, shapes, structures, and surface functionalities, for cancer therapy. Considering the particulate characteristics of noncoding RNAs, development of safe, stable, and highly efficient delivery systems for precision medicine has been one of the main tasks of nanomaterials research.

As described above, "genomics-driven therapy" is the essence of precision medicine. Tumor theranostics at the genetic level thus stipulates a fundamentally different assessment methodology for accurately predicting therapeutic efficacy in a clinical setting. In contrast, most of the current preclinical models fail to provide accurate therapeutic evaluation of cancer therapy. Recent studies show that PDXs are regaining popularity as preclinical models, especially when genome sequencing technologies have advanced to the point where the driver gene can be well identified. PDXs are developed directly from clinical samples without in vitro manipulation for the purpose of preserving the molecular heterogeneity and biological properties of cancer. PDXs are particularly useful, with integration of high-throughput techniques, to comprehensively characterize each model at multiple genetic levels, including mutation status, genetic structural alterations, and global gene expression patterns. Consequently, large panels of PDXs can be classified and selected based on genetic characterization and treated as cancer patient surrogates. Importantly, these genetic classification criteria in PDXs are highly adaptable to the representative clinical procedures in patient selection and targeted drug intervention. For instance, before clinical translation trials, an avatar of tumor patient with the same genomic molecular profile and physiology is found initially; PDXs are then verified to represent the oncogenomics and microenvironmental features of cancer. There have been an increasing number of studies on drug screening using the PDX models. Presently, however, lacking of driver-gene-based animal models has been the main reason for unsuccessful nanotherapeutics in clinical trials. Therefore, there is a critical need to develop more accurate animal models with well-screened oncogenomic mutation of cancer.

The concept of cancer precision therapy is schematically depicted in **Figure 1**. As shown in this figure, the patient is initially stratified into well-defined risk groups. The PDX panel is then established by direct implantation of tumor tissue from a group of cancer patients (for instance, head and neck squamous cell carcinoma: HNSCC) into immune-deficient mice (see clockwise blue arrows on the right side of Figure 1). To represent the subtype of a particular driver gene, the animal models developed must harbor the oncogene amplification that is selected from the PDX panel upon genetic characterization and classification. Accordingly, a type of small noncoding RNA (for instance microRNA-100) is specifically chosen for effectively regulating this driver gene (for instance fibroblast growth factor receptor 3 (FGFR3)). It is essential, as described above, to design a nanocarrier system capable of delivering the microRNA to cancer cells in vitro and regulating the driver gene-amplified PDXs in vivo. Critically, the delivery efficiency and therapeutic efficacy must be accurately evaluated by PDX models (see bottom block in the middle).

Yilong Wang is currently an Associate Professor at the Institute for Biomedical Engineering & Nano Science, Tongji University School of Medicine (China). He received his Ph.D. degree in applied chemistry from Shanghai Jiao Tong University in 2009. Before earning his doctorate, he studied at University of Washington in Seattle as an exchange student under the supervision of Prof. Younan Xia for one year. His main interest is biodetection using nanomaterials and devices, focusing on the early detection or prognosis of cancer and cardiovascular disease.

Zhiyuan Zhang is a Distinguished Professor at Shanghai Jiao Tong University. He is also an academician of Chinese Academy of Engineering and the director of National Research Center of Clinical Medicine for Oral Disease and Shanghai Key Lab of Stomatology. Currently, his research group is working in the field of establishment of personalized drug evaluation systems and development of novel targeted treatment strategies for head and neck cancer patients.

Donglu Shi is currently the Chair of the Materials Science and Engineering program at College of Engineering and Applied Science, University of Cincinnati. His research focuses on nanoscience-based precision medicine, cancer diagnosis and therapeutics, and biomaterials, which involve the design of unique material structures that not only interface with biological systems but also offer new biochemical–physical properties at the nanoscale for fundamental studies.

The design of the nanodelivery systems considers comprehensive parameters that are required for miRNA-based prognosis, synergistic RNA therapy, and biological barriers. The integration of the programmable nanosystems is accomplished by developing a variety of nanocarriers such as noble metallic, liposome, and polymeric nanoparticles (Figure 1). The physiochemical properties of the nanosystems include carrier sizes, particle surface functionalities, and their tumoral

microenvironment adaptation, all required and optimized in order to achieve synergistic delivery of noncoding RNAs, multiplex RNA detection, and prognosis (Figure 1). With novel nanostructure designs, the "one size fits all" nanocarriers will be replaced by their programmable counterparts that are specifically tailored to personalized-medical intervention for most predictable, accurate, and efficient cancer therapeutics. The concepts and principles shown in Figure 1 can be well extended and applied to other types of cancer precision therapy.

2. Organization of the Review

Considering the wide range of "Precision Medicine," we will focus on molecularly defined cancers and their nanotherapeutics via noncoding RNAs delivery. The gene delivery strategy is achieved by tailor-designed nanocarriers in the frame work of precision medicine. The so-called PDX is introduced as an effective assessment for accurate clinical predictability in targeted drug screening. The critical issues in nanomaterials selection and design for molecularly defined cancer theranostics are addressed by recent advancements in nano and medical technologies. Also discussed are the important roles of nanomaterials in gene delivery, oncogene regulation, PDX assessment of medical intervention, current challenges, and future opportunities.

The major steps of cancer precision therapy are summarized as follows: (1) identification of the oncogene that is genetically responsible for a class of common cancers; (2) efficient nanodelivery of noncoding RNAs that targets and regulates the oncogenetic expression; and (3) accurate assessment of RNA therapeutic efficacy via PDX that can address the issue of tumor heterogeneity.

Seven sections are presented here, respectively dealing with different critical issues involved in oncogene characterization, cancer diagnosis and therapeutics, and developing novel nanocarriers for cancer precision therapy. In Section 3, we introduce the basic concept of cancer precision medicine. In Section 3.1, we first provide an introduction to next-generation sequencing in a tutorial fashion for technical nonspecialists. This will give a baseline for precision medicine from the viewpoint of molecularly defined cancer, a new approach to personalized treatment. Molecularly defined cancers will be described in detail in Section 3.2 based on the current findings in medical science. Section 3.3 gives the definitions and descriptions of cancer precision therapy. The significance of new cancer classification will be emphasized in the highlight of genome sequencing technologies. The strength of noncoding RNAs delivery including microRNA replacement therapy will be introduced in Section 3.4. Patient-derived xenograft will be discussed with historical development and future aspects in Sections 3.5 and 3.6.

In Section 4, nanocarrier design for precision medicine is reviewed with regard to the most recent developments. In this section, criteria of nanocarriers for systemic delivery are described for cancer precision therapy. In Section 5, we discuss the important role of PDX model in evaluation of nanodelivery efficiency. Tumor prognosis via multiplexing microRNA nanodetection is introduced in Section 6. We finally conclude on all aspects of nanomaterials development for precision cancer therapy in Section 7.

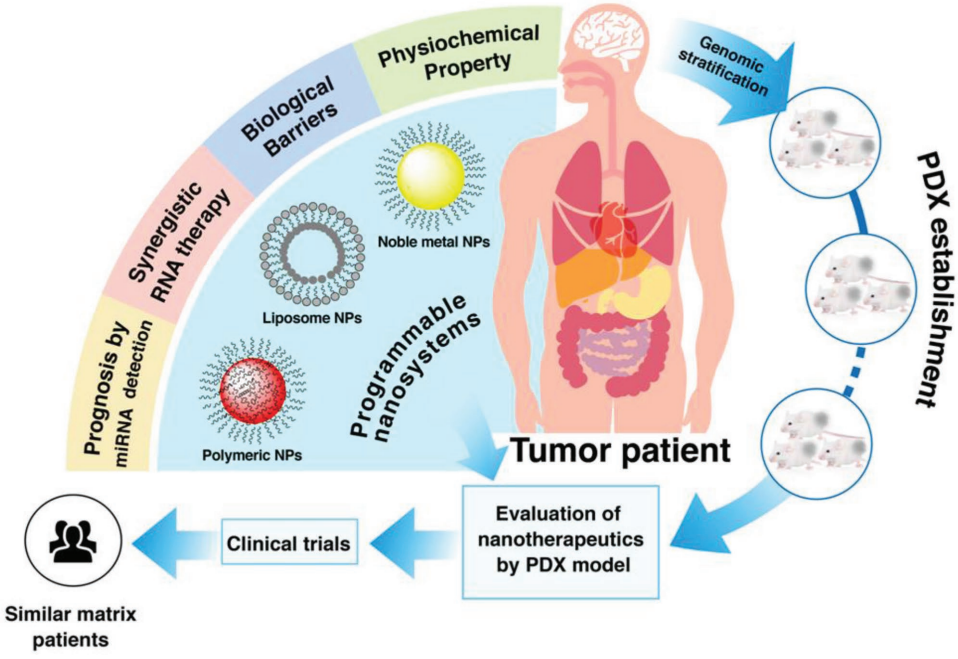

Figure 1. Schematic diagram showing the concept of nanomaterial-based cancer precision therapy.

3. Cancer Precision Medicine

3.1. Next-Generation Sequencing

Next-generation sequencing (NGS) is a general term that implies genomic sequencing, proteomic sequencing, epigenetic sequencing, and targeted sequencing.[9] It is particularly useful to acquire whole cancer genome with reasonable cost. Correlations between genomic sequencing (RNA sequence and transcriptional level, genetic mutation, DNA methylation, protein function and level) and clinical investigation (biological behavior, sensitivity/resistance to drugs, ending point) will enable identification of potential biomarkers for cancer precision therapy. In precision medicine, both validation of the biomarkers and stratification of patients are critically important in making the clinical treatment strategies. NGS has already entered "Omics Era" with miniaturized laboratory facilities allowing for testing thousands of molecules at a time.[10] With NGS, the Cancer Genome Atlas and the Cancer Genome Project have been established, based on genome sequencing and bioinformatics, to catalogue genetic mutations responsible for cancer, and to develop the landscape of cancer genome including point mutations, copy number variation, and translocations. Furthermore, potential driver genes and chromosomal structural rearrangements can also be identified via sequencing. These genomic events may affect a variety of cellular processes from cell signaling to metabolism to gene expression.[11] The clinical significance of NGS spans in all aspects of precision medicine that include diagnostics, prognostics, prevention, and treatability.[12] NGS plays an important role in finding potential biomarkers for predicting drug effects and stratifying patients into well-defined risk groups. By correlating NGS outcome to drug efficacy for a patient, predictors of therapeutics can be determined.[13] Upon validation of preclinical models, these biomarkers provide a highly valuable reference for physicians to make clinical decisions on prescribed therapy. There have been an increasing number of clinics that rely on NGS data for more precise diagnosis and therapeutics of cancer. Consequently, stratification for tumor patient based on the molecular characteristics is gradually emerging in clinical practice.

3.2. Molecularly Defined Cancer

Traditional classification of cancer is based on the tumor anatomic origins and the clinicopathologic characteristics, according to which the therapeutic strategies may be developed. However, distinct genetic variants cause heterogeneous clinical outcome and drug response may be heterogeneous even for those with similar histologic features and tumor stages, therefore resulting in poor predictability in drug response and prognosis of the patients.[14,15] Recent studies have shown that genetic aberrations of FGFR3, due to amplification, mutation, or FGFR3–TACC3 fusion, can function as an oncogenic driver in various types of malignancies including urothelial carcinoma, multiple myeloma, cervical carcinoma, and HNSCC.[16,17] These key findings have inspired the medical communities to consider a new cancer classification based on molecular characteristics of cancer as a preselective condition for precision and personalized treatment. The convergence of genetics, bioinformatics, and targeted therapeutics has all been rapidly expanding the scope of precision medicine by refining this new classification of cancer. Cancer classification in this fashion is therapeutically more relevantly based on molecular biology rather than traditional anatomic and histological criteria. Meanwhile, the currently advancing high-throughput technologies have enabled global gene expression and genomic and epigenomic analyses, presenting a new outlook of oncology.[18] Tumor heterogeneity has now been well observed in terms of genetic features and pathological characteristics, therefore demanding entirely different clinical prognosis and therapeutic strategies. As such, a new classification of cancer is emerging and technologically dependent on the advanced genome sequencing. Following this new classification, tumor treatment can be precision-targeted at an oncogenic mutation (driver gene) via various genetic regulatory strategies, for instance microRNA replacement therapy. However, other oncogenic mutations can exist that may not selectively respond to a particular therapeutic treatment or gene-regulation. Nonetheless, a single driver gene can be majorly responsible for prognosis of the tumor. Suppression of cancer can, therefore, be achieved by up- or down-regulating a single driver gene. This tumor behavior is sometimes described as "oncogene addiction" that provides a clinical base for cancer precision gene therapy.

The concept of new classification of cancer is schematically shown in **Figure 2**. As shown in Figure 2A, cancers arise due to acquisition of somatic alterations in their genomes that alter the function of key cancer genes, termed as the driver gene. But the tumors generated from different organs may share the same driver mutation and respond to the same therapy. For example, breast cancer (with HER2 alternation), gastric cancer, and colorectal cancer (or EGFR mutation) may all be found in non-small-cell lung cancer and head and neck cancer, sharing the same driver gene. A chosen microRNA (for instance, microRNA-100) can therapeutically be used to down-regulate this driver gene, delivered by nanocarriers. Multiomics can be applied in this process including genome, proteome, transcriptome, epigenome, and microbiome, as shown in Figure 2B, and combined into a set of omes in order to find the biomarkers efficiently. This will consequently lead to a coherent matching of genomic–oncologic relationship, as shown in Figure 2C.

3.3. Cancer Precision Therapy

A key problem in current cancer therapy is lacking of preclinical models that can reliably predict clinical activity of drugs in cancer patients, mainly due to tumor heterogeneity and genetic diversity. The conventional cancer therapeutics such as chemoradiotherapy is not based on the classification of cancer genetic alterations. Although cancer cell lines are originally derived from patient tumors, in vitro manipulation and genetic transformations will make the cell culture phenotypically homogeneous. In traditional cancer therapy, as shown in **Figure 3**, patients are often grouped according to the following: the anatomic regions where the tumors are found; the classification of malignant tumors (TNM classification, where "T" stands for the primary tumor site, "N" stands for the regional lymph node involvement, and "M" stands

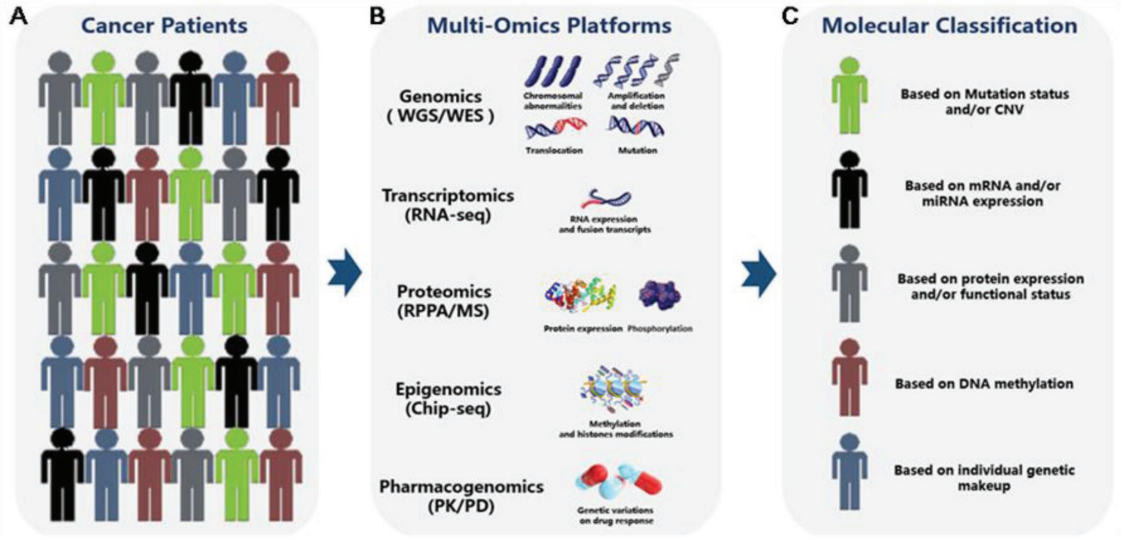

Figure 2. Schematic diagram showing molecular classification of cancer patients via NGS.

for the presence or otherwise of distant metastatic spread); histological grade; risk profiles, and clinical features. The therapeutic strategy normally follows a generalized protocol without consideration of tumor heterogeneity and genetic profiles. This type of conventional approach is often described as "one-size-fits-all" medicine, that may result in adverse effects for some patients.[19]

Precision medicine is targeted at an individual's genetic profile, also known as $N = 1$ (Figure 3). At the individual level, a specific genetic abnormality, or a driver gene can be identified, through genome sequencing, that is responsible for different cancers such as breast, lung, ovarian, colon, and some other cancers. With this approach, tumor diagnosis is taken to the next level with gene-mapping analyses, based on which the patients are categorized with specific driver genes. In this way, the treatment can be therapeutically applied more precisely by classifying individuals into subpopulations who respond to a specific treatment differently. The so-called "personalized treatment" is able to provide more reliable prognosis of the diseases that the individual patients may develop. It must be noted, however, not all oncogenes can be targeted and regulated by known microRNA and treatment options. Therefore, "precision medicine" is only a relative term, whose science is still involving. Furthermore, only part of the genomic landscape may be revealed from the molecular characteristics via immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and Sanger's method.

3.4. Noncoding RNA-Based Therapy

Noncoding RNAs are a class of varied RNA molecules that are not translated into proteins, and they play important roles in

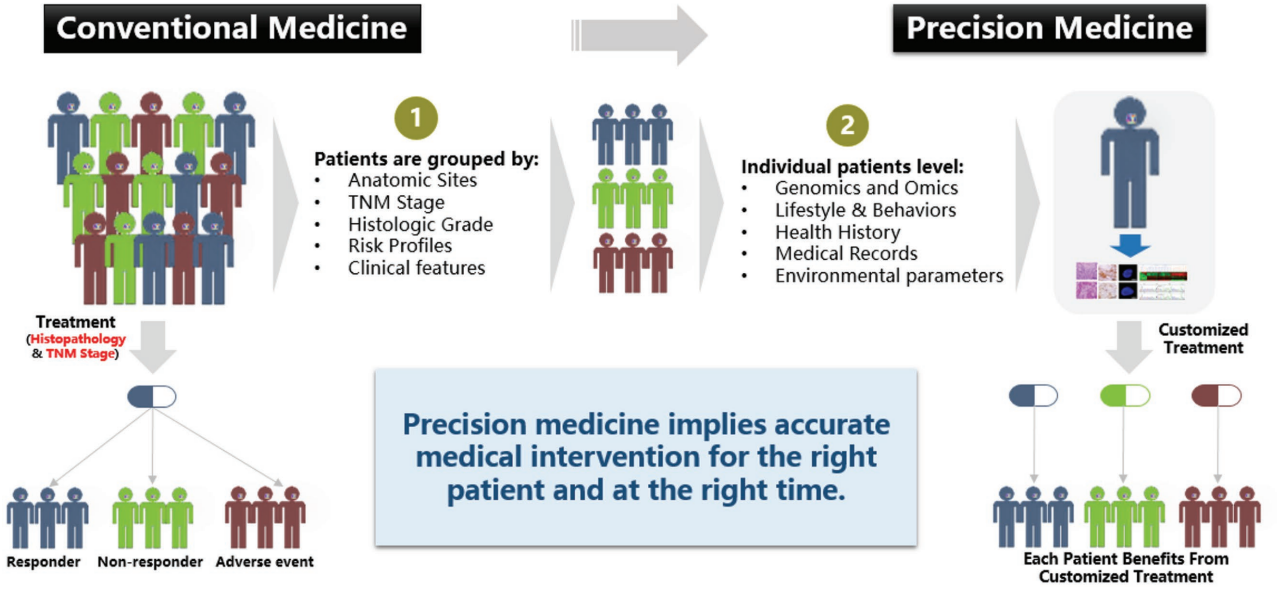

Figure 3. Schematic diagram showing the differences between the conventional medicine and precision medicine.

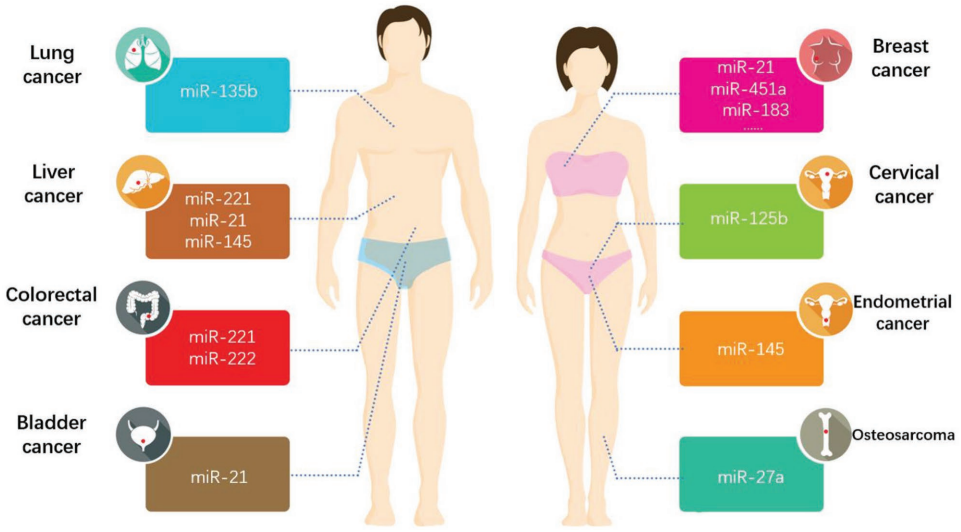

Figure 4. Schematic diagram showing different microRNA (miR) replacement therapies for various types of cancers.

tumorigenesis and progression. Among different noncoding RNAs, microRNA has been found to be a potential target. MicroRNAs are small, noncoding single-stranded RNAs that can regulate gene expressions via suppressing of their translation and stability.[20] In most of cancers, specific microRNA expressions can be up- or down-regulated, and dysregulation of some microRNAs has been found to be responsible for initiation, growth, and metastasis of cancer.[21–23] Although more than 99% of the approved drugs can target proteins for cancer treatment, a great many of proteins are found "undruggable." Regulating of microRNAs offers an effective alternative for targeted, precision medicine via specific manipulation of almost every protein population, including the "undruggable" proteins.[21] This unique gene regulation has shown promise for clinical therapy of several malignant diseases.[24] A recent study has shown that stratified glioblastoma, with the microRNA expression profiles, is divided into five clinically and genetically distinctive glioblastoma subclasses,[25] providing a clinical base for treatment strategy according to the state of microRNA in tumor tissue. Furthermore, circulating microRNAs are seen as new biomarkers in diagnosis, prognosis, and treatment of cancer, therefore able to not only serve as a noninvasive acquisition of tumor bioinformation but also as targets for drugs in cancer treatment.

Down-regulating of the amplified or overexpressed oncogenic microRNA in cancer is one of the emerging therapeutic strategies.[26] MicroRNAs are considered significant therapeutic targets for cancer therapy. They do not act alone, but exhibit their functions by forming RNA-induced silencing complex.[27,28] Miravirsen is the first anti-microRNA ASO, approved by FDA, and primarily used to deal with HCV infection, and tested successful in Phase II clinical trial. Small molecule inhibitors of microRNAs (SMIR) may be well delivered but rather difficult to degrade in vivo. SMIR can inhibit tumorigenic activity in cell lines and animal models. It can therefore enhance the effects of anticancer drugs compared to single drug application by inhabiting specific microRNA biogenesis. Another strategy is through reintroducing the tumor-suppressor microRNA into the tumor cells. Although alteration of microRNAs is diverse in different cancers, its expression is globally suppressed in tumor cells compared to healthy cells. Inhibition of specific microRNA may also enhance cellular transformation and tumorigenesis.[27]

The concept of microRNA replacement therapy is schematically shown in **Figure 4**. As shown in this figure, various cancers correspond to specific microRNAs. The first microRNA mimic was identified as MRX34 (microRNA-34) in the clinical test and developed by Mirnarx Therapeutic, Inc. (www.mirnarx.com). MicroRNA-34 is found to be a down-regulated tumor suppressor for numerous cancers. It can regulate more than 20 oncogenes, therefore, capable of treating multiple cancers. Synthetic microRNA-34 may exert as a potential therapy by transferring into cancers.[29] The major problem in microRNA-targeted therapy is, however, lacking of efficient delivery vehicles for transporting adequate microRNAs into cytoplasm without degradation.

3.5. Patient-Derived Xenograft in Precision Medicine

Drug attrition rates for cancer inhibition are much higher than other therapeutic areas. Only 5% of agents with anticancer activity in preclinical studies are licensed after demonstrating sufficient efficacy in phase III testing.[30] Analyses of attrition rate during 2011–2012 show that the medicine for oncology takes the highest proportion (nearly one third) of failures, among which the common objections are efficiency and safety.[31] The major problem attributes to lacking of preclinical models capable of recapitulating the heterogeneity of tumors in patients.[32] Preclinical models are important to provide an avatar of patient in clinical trials for predicting the drug efficiency. Drug efficiency assessments have shown significant changes of tumor cells in the wake of the culture process, including gain and loss of genetic information, alteration in growth and invasion properties, and loss of specific cell populations.[33] These results indicate incapability of the cell lines in recapitulating heterogeneity and microenvironment of the tumor in patients.

Patient-derived xenograft is a renewed tumor transplant model in immunodeficient mice, with the grafts being directly derived from fresh human tumors. The concept of the PDX model was developed in 1969 by Jørgen Rygaard and Carl O. Povlsen via subcutaneous injection of colon tumors into the immunocompromised mice. The resulting tumor in nude mice was found to grow much rapidly than the control mice, that marked the first usage of immunodeficient mice for tumor culturing.[34] Later, human tumor xenograft models were used to study the effects of radiation therapy and cytotoxic agent applied to patients, with successful correlations between the models and clinical findings.[35–37] The advantages of the PDX model have gradually been recognized as the accurate preclinical models in precision medicine. Using PDX, the xenograft can resemble the histopathology of the donator tumor and grow in the presence of an integrated stroma and tumor vasculature after several passages. No major changes exist between the donator tumors and their relating PDX model, except the gene-related stromal compartment and immune function.[33]

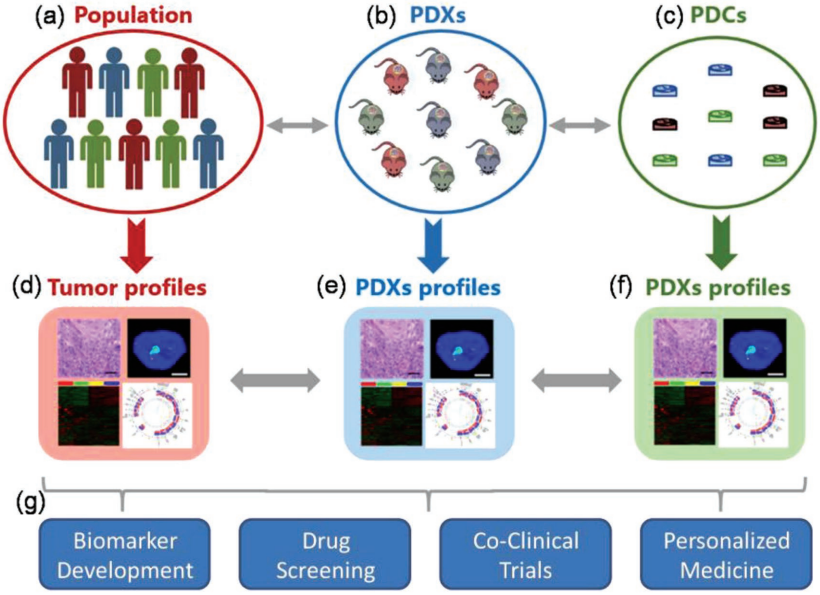

Figure 5. Schematic diagram showing the "one animal per model per treatment" approach in precision medicine.

PDX has been used to validate efficiency of the new anticancer therapeutics and the effects of new drug combinations. With current sequencing techniques, PDX is not only used as a model to passively verify the drug effectiveness but also an avatar of patients to predict prognosis, direct the therapeutic strategy, and instigate biological characteristics of tumor. In recent years, many trials have proved PDX a powerful model to advance cancer treatment in various ways. Many successfully correlated retrospective clinical findings to PDX data and led to high predictability on clinical outcomes. For example, cetuximab is an amonoclonal antibody of EFGR and approved by FDA on metastasized colorectal cancer and squamous cell carcinoma of the head and neck. Recent studies on cetuximab showed similar response rate in the PDX model of colorectal cancer and clinical findings.[38] The same results were found in other drugs such as bevacizumab and PARP inhibitor.[39] Furthermore, predictive markers were also found in the PDX models of colon cancer, with 84% (16/19) of the resistant tumors having activated mutations in KRAS, BRAF, and NRAS, among which KRAS mutation is 57% (11/19).[39] In clinical trials, patients with a colorectal tumor bearing mutated KRAS did not benefit from cetuximab.[40] Except mutations of KRAS, BRAF, NRAS, and PIK3CA (another protein in the downstream of KRAS), only a small proportion of cetuximab-resistant colorectal tumor showed quadruple-negative. In the quadruple-negative samples, HER2-amplification may not only be the negative predictor of cetuximab but also the positive predictor of the Pertuzumab.[41] In recent years, more of the PDX models have been designed in clinical trials for improved validation of the predictive genetic biomarkers, and relevant hypotheses in human clinical trial.

Despite significant progress, current PDX development is costly and time consuming with limited model numbers. Bruna and co-workers addressed this issue by combining PDX with short-term cultures of PDX-derived tumor cells (PDTCs). PDX/PDTC has proved to be a significant and viable platform for high-throughput drug screening.[42]

A novel therapeutic approach has been proposed, that combines with PDX models, patient-derived cells (PDCs), and NGS, to improve drug selection, search for biomarkers, and identify drug-resistance mechanisms. As shown in **Figure 5**a–c, PDXs and PDCs are established in the initial stage. Each patient is correlated to several PDXs and PDCs via their own tumor tissues. Upon drug screening, substantial numbers of PDXs and PDCs profiles are established (Figure 5d–f). High-throughput drug screening is then applied to PDCs that determines the tumor-sensitivity to potentially efficient drugs (Figure 5g). Selected drugs are correlated to the corresponding PDXs for optimization of drug efficiency in vivo. Once the optimized agent is selected, it will be incorporated into the therapeutic strategy for a particular patient in the so-called "personalized treatment." NGS will be applied on tumor tissues, xenografts, and PDCs before and after therapy. The genome sequencing profiles will be subsequently correlated to the clinical outcome in order to identify the potential biomarkers. Moreover, coclinical trials can also be carried out. In contrast to the drug selection process above, the potential drugs previously selected are applied to the patients and PDXs simultaneously in order to assess drug response in a real-time manner. In this way, the resistance mechanism and predictive biomarkers can be identified in the coclinical trial.

3.6. PDX with Well-Defined Driver Gene

Upon generating the PDX model, molecular stratification is the first step toward cancer precision medicine.[43] PDX is not only a tissue bank to expand the sample volume but also a platform for mining biological information. As mentioned above, tumors

such as breast cancer, liver cancer, and lung cancer have already been molecularly defined in several reports. To model heterogeneity of different tumors, genetic annotation of PDX must be carried out using high-throughput sequencing. Having collected all bioinformation (genetic mutation, protein function, the level of mRNA, methylation of the DNA sites, and so on) from the PDX generated, models are categorized based on existing criteria on human or genomic information. A model is accordingly selected for a particular targeted pathway. The selected model is used to search for a specific group of models for the best match of targets in large-scale screening. Upon administration of therapeutics, correlating genetic information with model outcome will enable identification of the predictive biomarkers for a specific drug or gene. This is also achieved by finding the genetic gap between the drug-resistant and drug-effective samples. Furthermore, the distinguished mutation can be investigated via evaluation of the molecular characteristics in response to the drug and downstream molecular activation. These results will provide the underlying mechanism for a specific drug resistance.

4. Nanomaterials for Cancer Precision Therapy

There have been many challenges in precision medicine that cannot be easily addressed by current medical methodologies, for instance, the targeted gene delivery. In the past decade, nanomaterials and nanotechnology have shown promise in meeting some of the key requirements in medical diagnosis and therapy, including cell targeting, medical imaging, drug/gene delivery, and a variety of therapeutic means based on chemical and physical properties at nanoscale such as magnetic hyperthermia and photothermal therapies. Although critical issues are yet to be fully addressed for these novel materials, a new era has begun in medical history that diagnosis and therapeutics can be well advanced via tailor-designed nanocarriers and their surface modifications.[44] In particular, special functionalities of these particulate carrier systems have been engineered capable of intelligent triggering of drug release, efficient gene delivery, sensitive biosensing, super-resolution nanoimaging, signal nanotransduction, and biological nanoanalytes. In the so-called nanomedicine, one of the major focuses lies in cancer diagnosis and therapeutics using unique nanostructures tailored to the specific biomedical and clinical applications.[45–49] In contrast to conventional materials such as bulk, single crystals, and thin films, nanoparticles are characterized with much smaller scales (≈10 nm), large surface areas, new structures, and properties associated with nanoscale, and a wide range of functionality, manipulability, and biomimic ability. As such, it allows for a great freedom in structural architecture, tunable properties, microenvironmental interactivity, nano-biointerfacial functions, and even the potential for developing nanorobots for special tasks at the cellular level, thereby exhibiting a promising future in both fundamental science research and clinical applications.

Cancer precision therapy has well utilized the unique nanoproperties and prompted generational development of various nanoparticles. In retrospect, the first generation (1G) nanomaterials are mainly composed of single, nonfunctionalized, and as-synthesized basic nanoparticles such as SiO_2, Fe_3O_4, ZnO, and quantum dots with limited properties and applications. To deal with the complex medical issues, nanoparticles must be functionalized with different moieties for diagnosis and therapeutics in a clinical setting, that include anticancer drugs, biological molecules, florescent dyes, tumor-specific ligands, and genetic entities. These components must be well assembled at nanoscale to function independently or cooperatively for the most efficient medical theranostics. As such, the term: "multifunctional nanosystem" has emerged as the representative second generation (2G) nanoparticles. Some systems at molecular level may be built with particular structures such as a tube or ring that can be driven by molecular interactions, which are sensitive to pH, light, electrical field, and "switched on–off" with structural reversal. A "microbivore" is an imaginative nanomachine which could function as an artificial white blood cell, or phagocyte. These future self-driven nanodevices may be classified as the third generation (3G) nanomaterials.

4.1. Nanocarriers for Noncoding RNA Delivery

Among all nanomaterials developed so far, we focus on those specially designed for precision medicine, more specifically, cancer precision therapy. The critical issues involved in cancer therapy deal with long circulating time of the nanocarriers, efficient targeting to the tumor tissue and cancer cells, high cell uptake and fast endosomal escape, large cargo loading and controlled release, and minimum toxicity via thorough body clearance.[50] A biological body may be regarded as a complicated closed system with a set of variable physiological parameters and microenvironment. Medical therapeutics applied to such a system will require "smart" nanocarriers with the "intelligence" level that is scaled to design complexity depending upon the specific strategies in precision tumor therapy.

Figure 6 shows a variety of nanomaterials that are commonly applied in medical theranostics, such as the inorganic and polymeric nanoparticles. One of the main tasks in nanodesign is to equip carriers with functionalities for overcoming the so-called "biological barriers" to which the carriers will first encounter in the blood stream. The typical biological barriers, as shown in Figure 6, include the mononuclear phagocyte system, intratumoral pressure at the extracellular matrix, and tumor cell membrane penetration that can sufficiently reduce the effects of the nanocarriers with payloads of drug and genes. Once delivered, these nanocarriers will have to be adaptive to the physiological environment for survival and efficient deployment of therapeutics. Upon interaction with serum and stroma in blood and tissue, some physiochemical properties of the nanocarriers will be altered and weakened, leading to invalidation of some biological effects. The "smart" nanosystems refer to a class of nanocarriers which can harness physiological cues for tumor cell targeting and desired release behaviors, such as activated cellular uptake or stimuli-responsive drug release.[51–56] The difference between the "smart" and "nonsmart" materials, as shown in Figure 6, is defined by its response to the biological barriers in vivo. The structure, size, and surface functionalization of the smart nanosystems all play important roles in overcoming these biological barriers, as

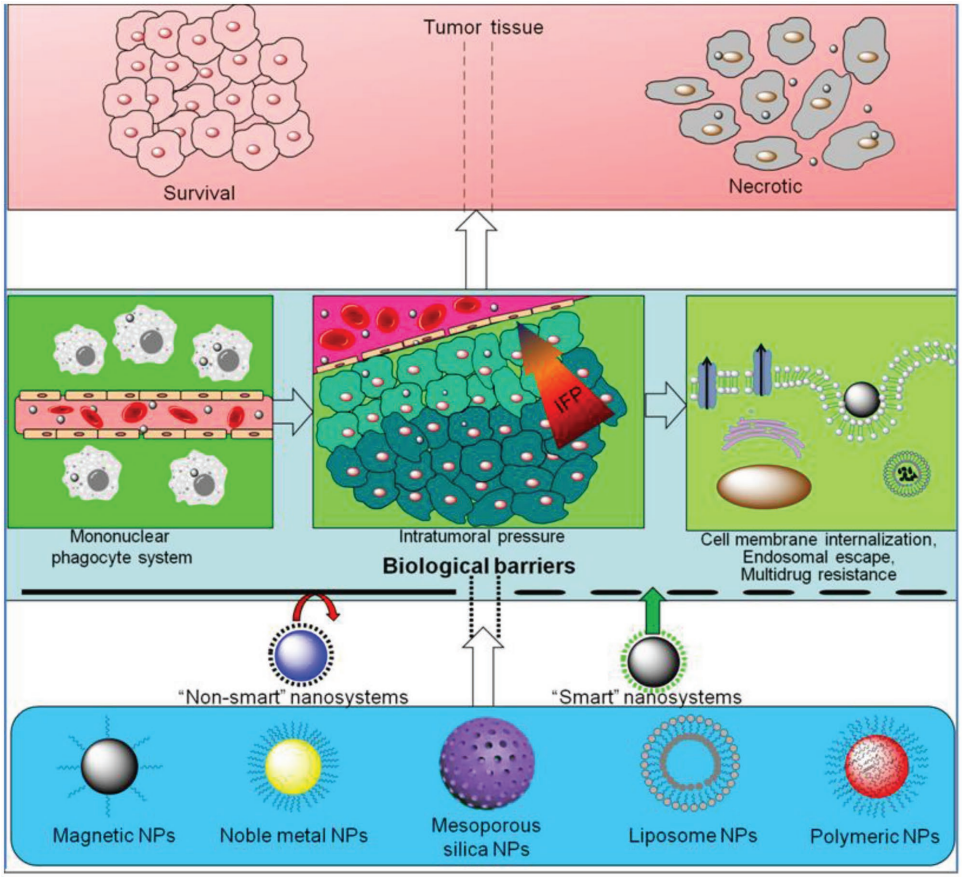

Figure 6. Schematic diagram showing various designs of "smart" nanosystems considering the in vivo biological barriers in cancer therapy.

indicated in this figure. Some of the typical "smart" nanosystems are introduced in the following sections.

The current advances in genomics and oncology have allowed for more personalized cancer therapy in a data-driven manner. Recently, new strategies of microRNA-directed cellular regulation by RNA interference (RNAi) utilizing exogenous triggers have emerged, including small interfering RNA (siRNA) or antisense oligomers.[57–60] The potential to regulate the expression of any cancer-relevant protein with comparatively high selectivity enables RNA-based therapeutics as a more effective and safe treatment in juxtaposition to traditional approaches. Clinical translation of this method depends, however, heavily on the design and development of the systemic delivery of nanocarriers.[61–64] Major considerations include the following: (1) In vitro data may not be reliable for carrier optimization especially when it is isolated from the cancer microenvironment in vivo; (2) the biological effects of the nanocarriers on the physiochemical features must be evaluated when interacting with living cells or protein in blood, and (3) precision therapy is only valid upon unambiguous therapeutic target at the gene or protein level. Combined therapy, utilizing the synergistic effects of nanocarriers with a variety of gene/drug payloads, has been an emerging strategy for improved antitumor efficacy. For instance, codelivery of gene/drug or gene/gene has been extensively investigated based on the synergistic properties of nanotherapeutics.[65] Among them, the tailor-designed hierarchical inorganic–organic composites are capable of efficient loading of two different cargos with intelligent sequential releases on targets.

4.2. Criteria of Nanocarriers for Systemic Delivery

For precision medicine, criteria must be first developed for nanocarriers in systemic delivery, based on all aspects of biological effects and basic medical requirements.[66,67] Cancer gene therapy is accomplished by inhibition of oncogenic microRNA or replacement of tumor suppressor microRNA, a unique and effective strategy to restore cellular homeostasis.[68,69] Oncogenesis, in part, has been associated with dysregulated expression of various microRNAs,[70–73] which exhibits some differences from small interfering RNA (siRNA) therapeutics.[74,75] The nanodelivery of nucleotides has shown significant advantages compared to administration of pure nucleotides. With nanodelivery, the stability of nucleotides is enhanced with more resistance to degrading enzymes and improved transfection of the therapeutic molecules into the cells. The delivery of nanocarriers can be further enhanced with optimized particle size and surface functionalization, characterized by specific tumor (cell) targeting, and self-triggered sequential responsive gene release. Despite extensive studies on various nanocarriers, a few focused on cancer precision therapy using the PDX models.

4.2.1. Nanocarrier Size

Among all nanocarrier design parameters, the hydrodynamic size is the most important one that strongly affects transfection efficiency, endocytosis, and toxicology of gene nanovectors in vitro or in vivo.[76–78] The guidelines for the hydrodynamic size consider the route of administration and biological barriers for in vivo gene delivery. The nanovector size is also critical in vitro for cell internalization and transportation inside the cancer cells. Regardless of chemical composition, an ideal size may assume a considerable range for gene delivery, particularly in overcoming certain biological barriers. For passive targeting of tumor via the enhanced permeability and retention (EPR) effect, macromolecules larger than 40 kDa may leak out from tumor vessels and accumulate in tumor tissues depending on the tumor type and the newly formed blood vessels.[79] The therapeutic effect can be dramatically influenced by the carrier size, therefore, critically controlled through different synthesis methods, among which, the template-assisted method has been widely employed in developing hollow or yolk–shell structured inorganic nanovectors.[80] A bioinspired templating method has been recently reported for preparation of lipid-bilayer vesicles with monodispersed sub-100-nm diameter.[81] In this approach, liposome self-assembly is nucleated and confined inside the rigid DNA nanotemplates. Using this method, homogeneous liposomes can be synthesized with four distinctive sizes.

To generate programmable lipid-like vehicle, the structural DNA ring can be constructed as a template. **Figure 7** shows the scheme for generating size-controlled vesicles by nanotemplating. The physiochemical properties of the liposomes are tuned through DNA-strand hybridization. A DNA-origami ring (red in Figure 7) is initially prepared to carry multiple single-stranded extensions (empty handles). The antihandles are prepared by chemical conjugation of liposome precursor, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamineN-[4-(p-maleimidophenyl)butyramide], to oligonucleotides with a complementary sequence to handles. Extra lipid and detergent are supplied to form liposome by hybridization of the empty handles and the lapidated antihandles (the green curl with orange head, as shown in Figure 7). The chemical structure of the lipidated antihandle is shown at the bottom of Figure 7. The desired product (liposomes, with their sizes determined by the DNA template) is purified via isopycnic centrifugation. At the final stage, the vesicles (the gray bubbles as shown in Figure 7) are released from the DNA ring template. The liposomes prepared in this fashion exhibit improved homogeneity compared to those by traditional methods such as extrusion.

Via size control, one is able to evaluate the size-related biological effects including circulation, internalization, retention, and overall antitumor efficiency resulting from a series of siRNA carriers with different diameters.[82] A typical example of size-related antitumor efficiency is shown in **Figure 8**. A series of cationic micellar nanoparticles (MNPs) with difference sizes of 40, 90, 130, and 180 nm are synthesized with similar physiochemical features and siRNA binding efficacies. As can be seen in this figure, suppression of MDAMB-231 cells is consistently correlated to different sizes denoted as MNP-(40, 90, 130, 180)/siRNA, among which MNP-180/siRNA (the largest size: 180 nm) exhibits the strongest intracellular green fluorescence signals in the cytoplasm compared to those treated with other sizes. As can also be seen in this figure, the MNPs of 90 nm (MNP-90/siRNA) show the most significant antitumor efficacy in the MDA-MB-231 xenograft murine model. This is attributable to the highest siRNA retention and best gene silencing efficacy following intravenous injection. These results suggest a size balance between

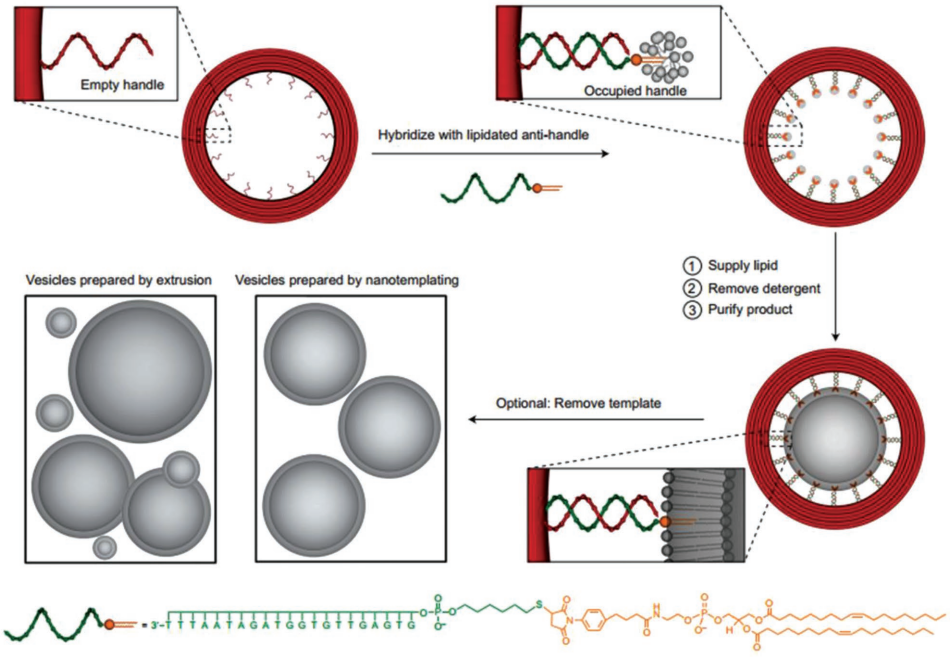

Figure 7. Scheme for generating size-controlled vesicles by nanotemplating. Reproduced with permission.[81] Copyright 2016, Macmillan Publishers Limited.

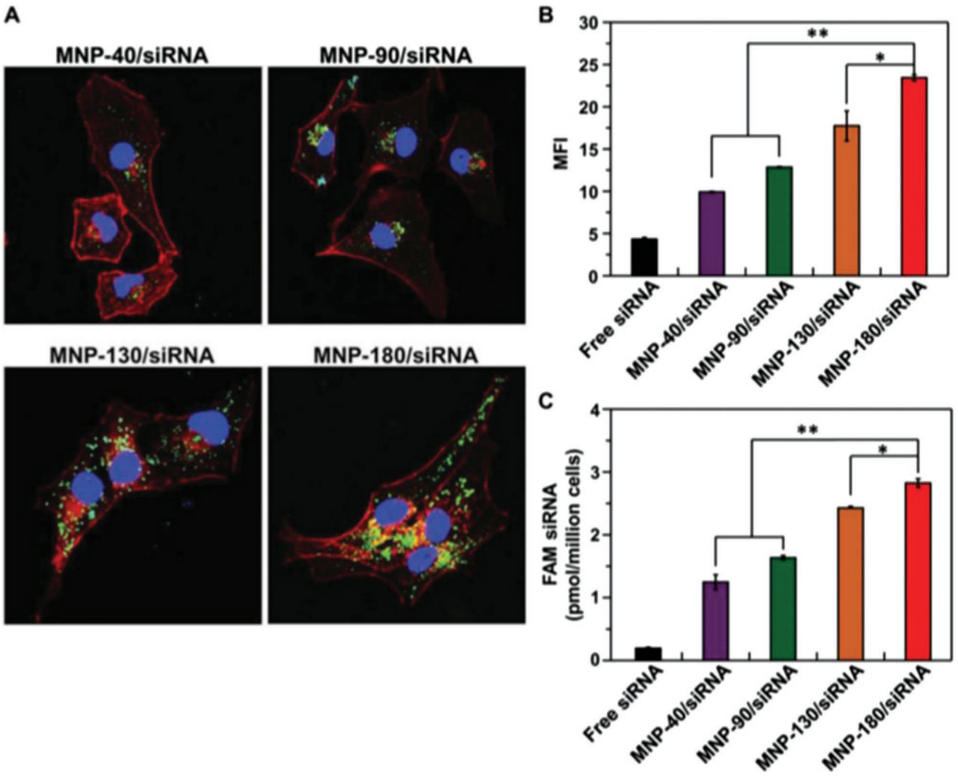

Figure 8. Therapeutic efficiencies achieved via nanocarriers with different sizes. Reproduced with permission.[82] Copyright 2015, Wiley-VCH.

retention of therapeutics within the plasma for treatment effectiveness and renal clearance for reduced long-term toxicity. Ideally, the nanovector size should be kept in a suitable range: small sizes in general facilitate long-term blood circulation, while considerable dimensions promote a high concentration of therapeutics at the tumor site due to pronounced EPR effect.

Figure 9A shows in situ nanoparticle size conversion (from small to large) via polyanion/PEI complexes, indicating excellent antitumor effect with negligible damage in major organs.[83] Dissociation of nanocarriers is therefore desired for more sensitive response to microenvironmental changes.[84] Wang and co-workers developed a nanovector that can dissociate upon arriving at the tumor site due to their pH-sensitive features.[85] Figure 9B,C are schematic diagrams, respectively, showing the pH-sensitive cluster nanobombs and related biological effects. To balance between the biological barriers related to the EPR effect and intratumoral pressure, the nanostructures can respond, in size, to different tumor microenvironment; starting with an initial size of ≈80 nm at neutral pH, then a dramatic size transition as pH reaches ≈6.5–7.0. As shown in Figure 9C, upon reducing to a dimension less than the dendrimer building block (10 nm), the loaded Pt drug is efficiently delivered into tumor tissue and cells. Wong et al. proposed a concept of multistage nanoparticle system with tumor-microenvironment-responsive characteristics, in order to compromise the requirement of tumor cell targeting.[86] A customizable multistage delivery vector was also developed, composed with biodegradable porous silicon, capable of encapsulating a variety of drug-loaded nanoparticles for enhanced tumor delivery.[87]

These bioinspired-delivery strategies have recently been further modified to include shape- and size-shifting nanoparticles based on DNA hairpin ligands, known as the "smart" nanomaterials that are adaptive to their local microenvironment.[88,89] Size-variable nanocarriers can modulate surface charges due to protonation of amino groups, triggered by acidic tumor extracellular environment. These smart nanocarriers are particularly useful for targeting and effective specific gene silencing in vivo.[90] Recently, Chen et al. prepared the shell-stacked nanoparticles with size and surface charge dual-transformable properties for enhanced tumor penetration and cancer cell uptake in deep tumor tissue.[91]

4.2.2. Surface Functionalization

One of the key features of nanoparticles is the surface functional groups conjugated for both structural architecture and medical theranostics. The nanovectors must be surface-functionalized for conjugation of various theranostic moieties in order to carry out required tasks including prolonged in vivo circulation time, precision recognition of tumor sites or tumor cells, reduced cytotoxicity, enhanced gene transfection, and improved biocompatibility. Artificial and natural polymers are often used to functionalize the nanoparticles for multiple functionalities. These include polyethylene glycol (PEG),[92] chitosan, polyethyleneimine (PEI), cyclodextrin, zwitterionic polymer,[93] and etc.[94,95] Some polymeric surface-functionalization can enable enhanced tumor cell targeting such as poly(glutamic acid) (PGA) and hyaluromic

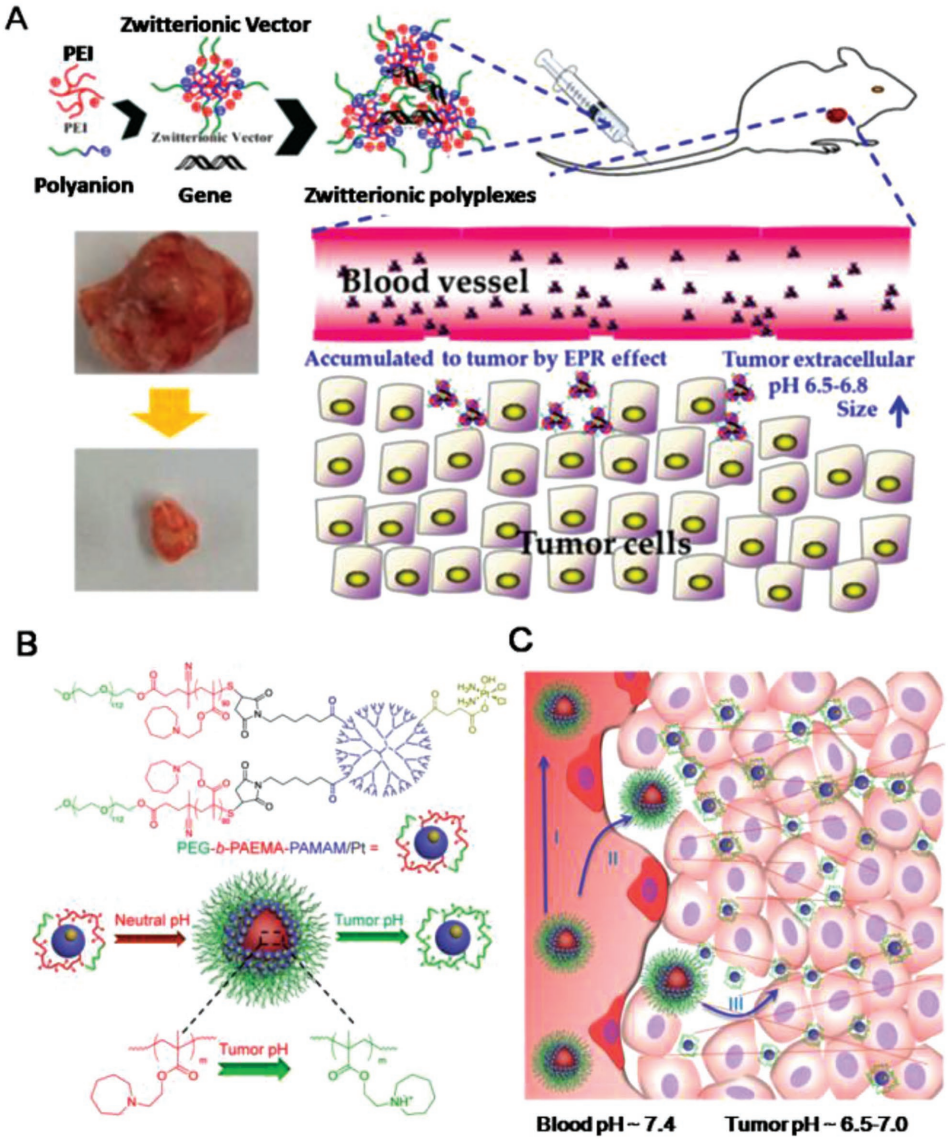

Figure 9. A) pH-triggered size increasing gene carrier for efficient tumor accumulation. Reproduced with permission.[83] Copyright 2017, American Chemical Society. B) Schematic illustration of the pH-sensitive cluster nanobombs and C) their corresponding biological effects. Reproduced with permission.[85] Copyright 2016, American Chemical Society.

acid (HA). The CD44 receptor-overexpressed cell lines were reported to be well-targeted through HA receptor-mediated endocytosis. This approach can be used to treat diseases in tissues with HA receptors, such as liver and kidney cancers. Layer-by-layer-assembled, cysteamine-modified gold nanoparticles/siRNA/PEI/HA complex shows enhanced target-specific intracellular delivery (**Figure 10**A). Without an inorganic core, siRNA/PEI-HA complex can efficiently target tumor cells (Figure 10B).[96–99] Pronounced tumor cell uptake has also been found with PGA receptor-mediated endocytosis.[100] Polymers can be assembled with multiple layers that facilitate gene transfection of nanocarriers in the presence of serum.[101] Biocompatibility, a key requirement in nanomedicine, also requires surface-functionalization with bioinspired, synthetic polymers.

Extracellular vesicles such as exosomes, are rather small with diameters of 30–100 nm, but representing an important tumor microenvironment. Communications within the tumor or between the tumor and stroma significantly influence tumor progression and interfere with treatment responses. Malignant cells rely on paracrine signaling, initiated from adjacent stromal cells, to resist anticancer therapies. Tumor-derived exosomes, related to tumor proliferation, angiogenesis, invasion, and premetastasis can be used for exosome-based cancer diagnosis and antitumor therapy.[102–104] Some investigations have been carried out on the endogenous identity of exosomes and interaction of cancer cells with gene-loaded exosomes.[105] Exosomes play crucial roles in protection and transport of endogenous biomolecules of microRNA as antitumor therapeutics.[106–108] Tasciotti and co-workers isolated cell membranes from

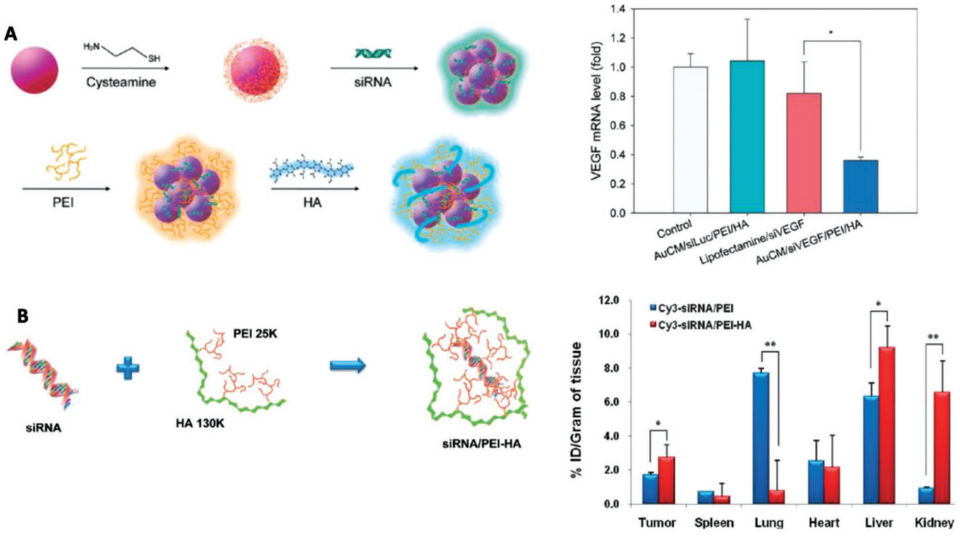

Figure 10. A) Schematic diagram showing the preparation of layer-by-layer assembled, cysteamine-modified gold nanoparticles/siRNA/PEI-HA complex and its enhanced delivery efficiency. Reproduced with permission.[99] Copyright 2011, American Chemical Society. B) Schematic representation of siRNA/PEI-HA complex and its improved tumor targeting ability. Reproduced with permission.[96] Copyright 2009, American Chemical Society.

leukocytes via particle coating to reduce opsonization and subsequent uptake by the mononuclear phagocyte system (MPS).[109]

Figure 11 shows a schematic diagram of the leuko-like vector and different interacting behaviors with a human umbilical vein endothelial cells (HUVECs) endothelial monolayer. The surface of (3-aminopropyl)triethoxysilane-modified porous silicon particles is coated with leukocyte-derived cell membranes through electrostatic and hydrophobic interactions (Figure 11A). Figure 11B,C, respectively, show the transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images of bare vector surface morphology, while Figure 11D,E is the TEM and SEM images of the vector with single layer of lamellar vesicles coating.[109] Stem cell membrane-coated nanogels can effectively reduce immune system clearance, therefore increasing the tumor targeting ability and antitumor therapeutic efficacy of doxorubicin-loaded gelatin nanogels in mice.[110] As is well known, virus RNA carrier exhibits high transfection efficiency, but at the cost of strong immunogenicity. Virus-like nanoparticles have therefore been developed as gene and drug carriers from different plant protein or Hepatitis B core protein.[111–114]

4.2.3. Overcoming Biological Barriers

Tumor targeting is well known for enhanced antitumor efficacy and reduced toxicity. One of the main requirements for cancer precision therapy is tumor cell targeting and internalization of the microRNA-nanocarrier complex. Some of the key

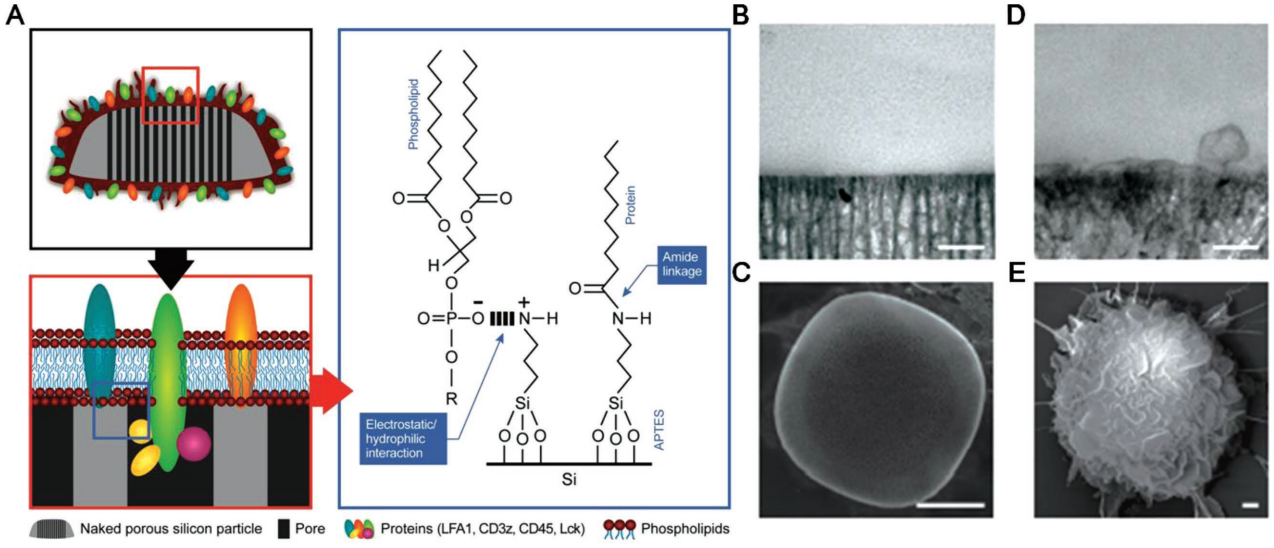

Figure 11. A) Schematic illustration of the leuko-like vector. B) TEM and C) SEM images of the bare vector. D) TEM and E) SEM images of the single layer of lamellar-vesicle-coated vector. Reproduced with permission.[109] Copyright 2013, Macmillan Publishers Limited.

issues deal with elimination, trapping, and destabilization of the nanocarriers, known as "biological barriers."[115] Nanocarriers can serve as a precision transport system that delivers the cargo to a specific target in vivo.[116] Although heterogeneity of the biological barriers exists from patient to patient, and from lesion to lesion, some general principles apply for nanocarrier designs.[117] The typical barriers include clearance by MPS, hemorheological/blood vessel flow limitations, pressure gradients at the tumor stroma, cellular internalization, escape from endosomal and lysosomal compartments, and drug efflux pumps. Several studies have shown that the EPR effect-mediated passive tumor targeting is effective in overcoming some biological barriers for certain types of tumors.[115] Special nanocarriers need to be developed for specific tumor models at a given angiogenic phase.[118–120] Depending on the method of administration, nanocarriers must adapt to the different biological barriers in vivo. Nanocarriers are normally I.V. injected in the blood stream, and likely encounter barriers such as MPS in liver, tumor tissue, cancer cells boundary, subcellular organelle, and drug resistance related protein. To escape from phagocytic clearance, the conventional method relies on PEGylated-surface functionalization. In addition to optimization of the carrier physiochemical properties, such as size and surface functionalization, biomimicing is another effective strategy. For instance, blood brain barrier (BBB) is a major obstruct to drug delivery for brain tumor therapy. Recently, neutrophil-carrying liposome has been used to deliver chemodrug to brain tumors via penetration of BBB by increased inflammatory factors at the tumor site.[121] The biomimic-membrane-modified nanocarriers show well improved biocompatibility and low immunogenicity.

4.2.4. Hierarchical Structure and Ordered Functional Moieties

Significant progress has been made on targeted antitumor therapy via controlled assembly of nanosystems with synergistic bioeffects.[122–124] The current effort on synergistic delivery has gradually shifted from random diffuse of drug/gene from carriers to more orderly controlled release by unique nanostructural architecture and multifunctional moieties. For "smart" gene delivery with high temporal–spatial precision, various bioinspired strategies have been developed for physiological and environment responsive targeted gene delivery.[125] To deal with chemodrug resistance of tumor cells, extensive studies have been focused on the development of RNA/drug codelivery nanosystems.[126–130] Some nanosystems are designed with physiological-condition-triggered self-assembly or disassembly abilities. Among them, gold nanoparticles have been widely studied for its unique thermos physical properties.[131,132] Zhang and co-workers developed the thermosensitive-polymer-modified gold nanoshell composite nanoparticles as a gene/drug nanocarrier. MicroRNA inhibitor and chemodrug can be carried separately within the polymer surface and gold nanoshell. In this way, the cargos can be released in a sequential order for enhanced therapeutic efficacy, specially designed to overcome drug resistant. The drug release is controlled through dissolving the gold nanoshell by photothermal heating (**Figure 12**).[133] The lipid-layer-coated mesoporous silica can also sequentially release two drugs, in order, by a time-dependent diffusion process.[134] Paulmurugan and coworkers synthesized antisense-miR-21 and antisense-miR-10b loaded PLGA-*b*-PEG polymer nanoparticles and performed a targeted codelivery of antisense-microRNAs to triple negative breast cancer through sustained release. The subsequent synchronous blocking of target microRNAs by two cargoes with different bioactivities prove to be an efficient strategy for targeting metastasis and antiapoptosis in the treatment of metastatic cancer.[135]

Among these well-designed polymeric gene nanocarriers,[136,137] the versatile and hierarchical surface functionalization of inorganic nanoparticles, with synergetic effects of cargos, show high potential for efficient gene delivery.[80,138] Some typical examples include codelivery of two cargoes aiming at clear cancer therapeutic target, in which the bioeffect of one cargo is the trigger for the other. Furthermore, codelivery of two genes requires coloading of siRNA and microRNA in one nanovector.[139,140] The strength of two different noncoding RNAs, codelivered for cancer therapy, lies in the synergetic effect from specifically silencing expression of cancer-related genes and/or regulation of the pathway for cancer development and progression.

4.3. Nanocarriers for Cancer Precision Therapy

In precision gene therapy, delivery of therapeutics via nanocarrier design is crucial especially when aiming at a specific epigenetic alteration.[141] Personalized genomics data is essential for customized gene delivery and effective pharmacokinetics. Precision medicine therefore demands complex medical protocols, advanced genome sequencing analysis, and ultimately the selection and design of nanocarriers. The advanced nanomaterials and nanotechnology, development of gene sequencing technology, emerging biomarkers, and targeted therapeutics against dysregulated epigenomes in cancer cells will have to be all combined for realization of precision medicine, especially in a clinical setting.[142] Several microRNA overexpressions associated with some cancer types have been discovered recently, such as microRNA-155[143] and microRNA-21[144] which can be used as the therapeutic targets in cancer precision therapy.

4.3.1. Emerging Biomarkers for RNAs Nanotherapeutics

Using new genomic sequencing technologies, such as NGS, more information on genomics from clinical patients with different anatomic features have become readily available, which is extremely valuable in clinical treatment of cancer.[145,146] The "omics" technologies have been utilized to investigate the signaling pathways and biological networks, and identify the pathway mechanisms. However, the large amount of data generated makes it difficult to distinguish between the causative mechanisms.[147] For example, recent deep sequencing of HNSCC genomic landscape reveals a multiplicity and diversity of genetic alterations in malignancy.[148,149] Among them, the PI3K/mTOR pathway is reported to be most frequently

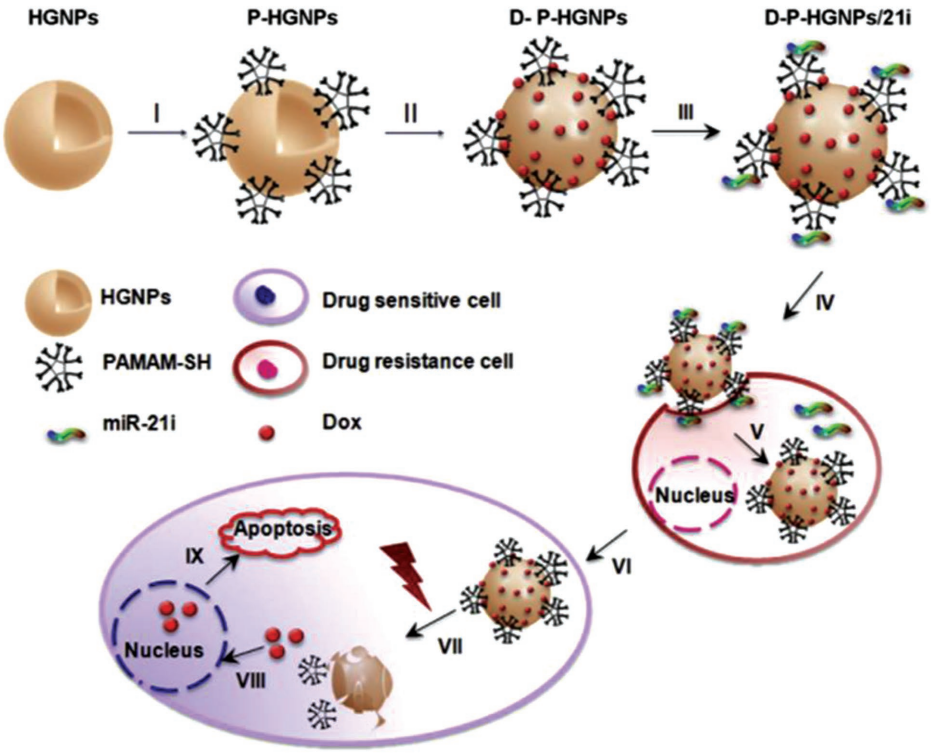

Figure 12. Schematic illustration of the sequential and synergistic codelivery vectors. Reproduced with permission.[133] Copyright 2016, Elsevier.

activated, and plays a central role in cancer initiation and progression. In this way, targeting of mTOR may present a possibility of precision therapeutics for HNSCC.[150] Clinical trials have shown encouraging results that mTOR inhibition exerted potent antitumor activity in the HNSCC systems.[150] Patients with lung adenocarcinoma showed pronounced responses to agents that target mutant BRAF in melanomas or mutant ALK. These findings indicate a genomic–oncologic relationship via driver gene regulation leading to subsequent cancer suppression, which is a quintessential example of precision medicine.[151] It should be noted that activating mutations in EGFR and ALK fusions are typically not present in lung squamous cell carcinoma (SQCC).[152] While molecularly targeted agents are now available for lung adenocarcinoma, no effective counterparts have yet been developed specifically for lung SQCCs. Studies on therapeutic targets for lung SQCC show that 96% (171 out of 178) of tumors contain one or more mutations in tyrosine kinases. These observations provide possibilities for targetable gene or pathway alteration in lung SQCC.[153] Correlations have been developed between clinical findings and molecular characteristics in breast cancer (divided into four types, luminal A, luminal B, HER2- type, and triple negative), glioblastoma multiforme (divided into three types, categorized by EGFR, NF1, and PDGFRA/IDH1), and colorectal cancer (divided into four types, CCS1-4 divided by a group of molecular characteristics).[154,155] Although the subtypes are not divided precisely according to the driver gene, they give much better predictability in prognosis and drug response to therapy compared to traditional cancer classification.

4.3.2. Targeted Noncoding RNAs Nanotherapeutics

The main cancer therapeutic modalities include surgery, radiation, and chemotherapy. As is well known, these conventional treatments not only pose high risks of high morbidity and mortality but also serious systemic toxicities. With individual genomic information on oncogene mutation of the stratified patient, novel druggable targets for noncoding RNAs therapeutic interventions in various human malignancies present overwhelming advantages. Recently, the driver-gene-based precision therapy is fast emerging utilizing the "-omics" technologies. Some advanced delivery nanosystems, such as liposome nanoparticles, have been developed with improved biocompatibility and delivery efficiency.[156] Huang and co-workers developed LPH nanoparticles (liposome-polycation-hyaluronic acid) for delivering siRNA and/or miRNA with a single formulation capable of simultaneously targeting several oncogenic pathways including c-Myc/MDM2/VEGF in lung metastasis. Tumor-specific scFv was conjugated onto the surface of nanoparticles for enhanced cancer cells targeting. Their results showed successful inhibition of c-Myc, MDM2, and VEGF protein expressions leading to significant suppression of B16F10 metastatic tumor growth.[140] With the similar nanoparticle formulation, Wang and co-workers used cyclic arginine-glycine-aspartic (RGD) peptide-modified LPH nanoparticles to deliver anti-miRNA antisense oligonucleotides for down-regulating the target microRNA in human umbilical vein endothelial cells.[157] Pan and co-workers[158] developed cationic lipid nanoparticles to deliver premicroRNA-107 as an anticancer therapeutics in HNSCC. They found pre-miR-107 well delivered into HNSCC cells by greater than 80 000-fold compared to free pre-miR-107. Previous in vitro

and in vivo experiments have also shown effective suppression of tumorigenesis of HNSCC by RNA nanotherapeutics as a result of specific targets: protein kinase Cε (PKCε), cyclin-dependent kinase 6 (CDK6), and hypoxia-inducible factor 1-β (HIF1-β).

5. PDX Model for Evaluation of Nanocarriers Delivery Efficiency

There has been an increasing need to apply PDXs as preclinical models in cancer precision therapy. These models are developed without in vitro manipulation therefore preserving the molecular heterogeneity of cancer. Furthermore, the advanced high-throughput techniques enable characterization of each PDX in terms of mutation status, genetic structural alterations, and global gene expression patterns. PDXs can be classified and applied as viable cancer patient surrogates. Considering cancer molecular heterogeneity and microenvironment, recent research has been increasingly focused on evaluation of therapeutic effects based on the PDX animal models.[159–164]

In RNA therapy, the tumor animal model is a particularly important factor which can be easily overlooked. Cell-derived xenograft has been extensively used to evaluate the therapeutic effects of systemic RNA delivery. Yang and co-workers[165] established an orthotopic human pancreatic cancer PDX model in SCID and nude mice to study the induction of tumor cell death by the theranostic nanoparticles carrying a chemotherapy drug doxorubicin, targeted at insulin-like growth factor 1 receptor (IGF1R). The research has, for the first time, applied the PDX models to evaluate the therapeutic outcome according to the oncogene instead of anatomic organs, and therefore paved a new path in cancer precision therapy through oncogenically defined patient.

A proof-of-concept study on microRNA replacement precision therapy has also been recently reported via development of nanodelivery, targeting at FGFR3 oncogenic alteration using the PDX model.[166] The strategy of this work has provided a rationale for precision medicine, as described as follows: (1) Identification of the oncogene for FGFR3-driven tumors by genome sequencing; (2) design of a nanovector delivery system for in vivo microRNA delivery via a facile and reproducible process; (3) efficient microRNA delivery predominately targeting at FGFR3, and (4) down-regulation of the FGFR3 pathway by microRNA with PDX assessment in vivo.

For gene delivery in this comprehensive study, a nanovector is developed with microchannels hierarchically functionalized with ternary polymers for high microRNA payload.[167] For biocompatibility and colloidal stability, the spherical nanovector is further functionalized with the negatively charged polyacrylic acid molecules.[167] This uniquely designed nanosystem has effectively delivered microRNA to the tumor site and down-regulated the driver gene for patients harboring FGFR3 aberrations, presenting a classic example of cancer precision therapy.

6. Multiplexing MicroRNA Nanodetection for Tumor Prognosis

The level of intracellular microRNA in gene therapy is one of the most critical indicators not only for microRNA replacement therapy but also for down-regulation of endogenous microRNA by antisense oligomers. Multiplexed detection of microRNA can provide complementary information for clinical decision-making and evaluation of therapeutic outcome. Many methodologies have been developed for multiplex or single microRNA detection based on various mechanisms,[168] including dye-quencher cyclic enzymatic amplification method,[169] microfluidic-paper-based analytical devices,[170] donor–acceptor Förster resonance energy transfer (FRET),[171] FISH fluorescence imaging,[166,172,173] cascade hybridization reaction,[174] and solid-state biosensors.[175,176] Most of these methods are developed for early diagnosis or prognosis of diseases according to their sensitivity, specificity, type of tissue or cellular samples. Prior to the emergence of nanotechnology, the conventional methods for microRNA detection include reverse transcription polymerase chain reaction,[177] Northern blotting,[178] and microarray technologies.[179] Due to limited microRNA in total RNA samples and the vulnerability to degradation, these conventional methods have intrinsic limitations in detection sensitivity and specificity, especially for multiple analyses.

Nanomaterials have been utilized for highly sensitive and specific microRNA detection. Quantum dots and graphene oxide have widely been used in the donor–acceptor Förster resonance energy transfer method.[171,180–182] The detection selectivity of these methods is, however, heavily dependent on the ratio of signal amplification to background fluorescence. Surface-enhanced Raman scattering (SERS)-based sandwich hybridization assay has been investigated for target RNA to form hybrid with the nanoprobe RNA.[183] Upon specific RNA–DNA hybridization, monoclonal antibody conjugated Au nanoparticles with high affinity to RNA have displayed a signal amplification effect based on surface plasmon resonance enhanced light scattering.[184] The log of odds (LOD) can be as high as 60×10^{-15} M when using microRNA-122 as the model. The high detection sensitivity can even be realized with significant background of nontarget RNAs. In quantum dot-labeled strip biosensor, LOD of microRNA-21 detection of cell extraction sample can reach as high as 100×10^{-9} M, by an addition of target-recycled nonenzymatic amplification procedure.[185] The photoflorescence decay is related to the distance between the dye Tb and quantum dot nanoparticle, another critical parameter for specific detection of microRNA strand.[186]

However, few studies have so far been carried out on in situ microRNA detection for prognosis of cancer treatment by noncoding RNA delivery. Considering the inconsistencies between the spiking samples and RNA extraction efficiency from different labs, the quantitative detection of microRNA in living cells deserves rigorous investigation with advanced nanotechnologies. With the cascade hybridization of RNA strand, microRNA-21 in living HeLa cells have been directly imaged by a method using two programmable nucleic acid sequences, labeled with either a donor or an acceptor fluorophore dye.[174] A unique design of hairpin stem has been developed with the controlled loop lengths of nucleic acid sequence. Hairpin stem internal mismatch is intended for compromising the stability of the hybridization product in physiological environment. The difference between hairpin stem approach and other microRNA detection is that the former does not require any purification of target during the

process, a critically important advantage for diagnostics or prognostics of cancer.[187]

MicroRNA detection in living cells can also be achieved by using a sensitive method based on biodegradable MnO_2 nanosheet-mediated signal amplification.[188] The tumor redox-sensitive MnO_2 nanosheet has been used to carry and quench the dye-labeled hairpin probes. Upon target cancer cell endocytosis, the two-dye labeled probes are released from MnO_2 carriers, triggering cascaded assembly of two hairpins, and subsequently outputting significantly enhanced FRET signal. These hybrid microRNA detection nanoprobes show promise in real-time monitoring of the prognosis of noncoding RNA therapy with an extremely high sensitivity. Hybrid graphene oxide gold nanoparticles exhibit 1×10^{-15} M LOD in the surface plasmon resonance (SPR) process, particularly useful for microRNA detection. A sandwich-like DNA hybridization can target specific DNA molecules. Au films, covalently functionalized with thiolated DNA nanoprobes, and modified Au probes, linked on the grapheme oxide sheets, have been the recently developed microRNA sensors.[189] The GO-Au-SPR biosensor does not require any use of special capture probe, which is an expensive kit with toxic chemicals.

7. Summary

One of key contributions of nanotechnology to cancer precision therapy is the ability to deliver noncoding RNAs that can target and regulate specific oncogenes, responsible for a class of cancers. Nanodelivery of noncoding RNAs has proved to be a powerful therapeutic strategy for "oncogene-driven" cancer patients. This approach is clinically viable and adaptable to other types of molecularly defined cancers for personalized treatment. In cancer therapy, the PDX model is becoming widely recognized, and used as the avatar of cancer that preserves the genetic characteristics and microenvironment of the patient. The "intelligent" carriers have been tailor-designed for all aspects of precision medicine based on a variety of polymeric, inorganic, and bioinspired nanomaterials. The interdisciplinary collaborations between medical and materials researchers have been the key to the current success in jointly dealing with complex issues in cancer precision therapy. With further progresses in nanotechnology, the therapeutic carriers will be able to target the tumor tissue and cells in vivo in a precision and microenvironment responsive fashion. Today, more versatile nanocarriers are being designed and developed tailoring to the specific, personalized, customized cancer therapy in a clinical setting.

In addition to the PDX mouse models, large biobanks will be built with relevant patient information on a variety of tumor types for systematic drug screening. With the advances in precision medicine, the human tissue models will also be developed for cancer research, with elimination of the long-standing cultural reliance on animal models.[190] Furthermore, tumor organoids is another consideration as preclinical cancer models.[191,192] 3D organoids reflect the natural state of organs more precisely in juxtaposition to the traditional cell culture models.[193] Without standard PDX animal models, the efficacies of different delivery systems may not be accurately and comprehensively assessed and compared. It is, therefore, urgent to develop biobanks with more complete patient medical information and computational tools. More "intelligent" nanocarriers will have to be designed capable of dealing with complex biological and genetic systems.

Medical science has been traditionally relying upon three major fields, namely, anatomy, physiology and biochemistry. Nanomedicine is probably the most important emerging field since the dawn of nanotechnology. It is the new discovery of a variety of unique nanomaterials that makes medical diagnosis and therapeutics possible at the cellular and genetic levels. Based on tailor-designed nanocarriers, the drug and gene delivery can now be spatially and temporally controlled, delivered, and released in a highly intelligent and precision fashion with minimum toxicity. Consequently, medical science has entered upon a new era especially when more accurate theranostic tools become available. Combined with advanced nanomaterials and biotechnologies, medical science will soon reach a new level of precision theranostics in a clinical setting.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biological barriers, genome sequencing, microRNA, nanomaterials, oncogenes, patient-derived xenografts, precision medicine, tumor microenvironment

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