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Realizing Cancer Precision Medicine by Integrating Systems Biology and Nanomaterial Engineering

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Many clinical trials for cancer precision medicine have yielded unsatisfactory results due to challenges such as drug resistance and low efficacy. Drug resistance is often caused by the complex compensatory regulation within the biomolecular network in a cancer cell. Recently, systems biological studies have modeled and simulated such complex networks to unravel the hidden mechanisms of drug resistance and identify promising new drug targets or combinatorial or sequential treatments for overcoming resistance to anticancer drugs. However, many of the identified targets or treatments present major difficulties for drug development and clinical application. Nanocarriers represent a path forward for developing therapies with these "undruggable" targets or those that require precise combinatorial or sequential application, for which conventional drug delivery mechanisms are unsuitable. Conversely, a challenge in nanomedicine has been low efficacy due to heterogeneity of cancers in patients. This problem can also be resolved through systems biological approaches by identifying personalized targets for individual patients or promoting the drug responses. Therefore, integration of systems biology and nanomaterial engineering will enable the clinical application of cancer precision medicine to overcome both drug resistance of conventional treatments and low efficacy of nanomedicine due to patient heterogeneity.

1. Introduction

Cancer precision medicine is an approach that matches the most appropriate treatment with the maximum efficacy to individual cancer patients by analyzing their genomic and molecular profiles. Biologists and physicians have defined specific genetic alterations or abnormal gene expression patterns as biomarkers that match with particular cancer-targeted therapy. For instance, breast cancer patients with excess abundance of the human epidermal growth factor receptor 2 (HER2), with or without amplification of the erythroblastic oncogene B 2 (*ERBB2*), are prescribed trastuzumab, an antibody that targets HER2.[1]

Although the recent clinical trial studies represent innovative approaches to cancer precision medicine, only 11–23% of the patients experienced objective responses.[2–4] This shows that simply matching molecular alterations with currently available targeted therapies is insufficient. One reason for the low rate of objective responses in precision medicine is due to resistance of the cancer cells to the targeted therapy.[5] Intrinsic and acquired drug resistance arise through complex compensatory regulation and hidden dynamics of the biomolecular network within a cancer cell. Systems biology contributes to unraveling these hidden mechanisms of the biomolecular network, predicts counterintuitive outcomes of drug treatments, and identifies novel drug targets and treatment regimens that can overcome drug resistance. However, the treatments suggested by systems biological approaches are often very challenging to be implemented with conventional drug delivery systems and instead require genetic manipulation, targeted delivery of bioactive molecules, or controlled simultaneous drug administration at a specific site. Thus, a different type of delivery system, such as nanocarriers, is highly needed.

Nanomedicine is an application of nanomaterial engineering to medicine to facilitate disease diagnosis, enable targeted drug delivery, and provide imaging capabilities. Many approved or investigational nanodrugs are nanocarriers that encapsulate drugs approved for use in other modes of administration or formulation.[6] The characteristics of a nanocarrier, such as its size, release kinetics, and surface chemistry, have the potential to enable nanodrugs to better reach the targeted tumor site and efficiently release drugs in a specific sequence or combination. Thus far, compared to conventional formulations of the encapsulated drugs, most approved nanodrugs have reduced toxicity but do not improve the efficacy of the response.[6] This low efficacy results from heterogeneity of cancer patients, which causes insufficient drug accumulation in tumor, unexpected interactions between nanoparticles and biological molecules, and the individual patient being resistant to encapsulated drugs.[7]

Recently, personalized nanomedicine may overcome the low efficacy problem by considering the heterogeneity of cancers.[8–10] Cancer patients are heterogeneous in terms of their genetics, transcriptomics, epigenetics, and tumor

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microenvironment (TME). Additionally, different tumors have different permeabilities and retention of nanoparticles in different sizes and compositions, which is referred to as heterogeneity of "enhanced permeability and retention (EPR)."[11] Not only are there differences between cancer patients, but the cancer cells themselves within a patient can be heterogeneous. Due to this complexity, there are still many challenges remaining. We suggest that systems biology can contribute to resolving these problems related to cancer patient and cancer cell heterogeneity and achieving personalized nanomedicine.

Herein, we present studies from systems biology that demonstrate how this approach can help resolving drug resistance by identifying novel drug targets and combinatorial therapies. We then present nanomaterial engineering studies showing how such outcomes of systems biology can be implemented with nanocarriers. Moreover, we also show that systems biology can help overcoming some critical challenges of personalized nanomedicine, such as promoting EPR and selecting appropriate payloads. We propose that integrating these two distinct fields of study will successfully overcome the challenges of each field and effectively achieve precision medicine for treating complex diseases such as cancer (**Figure 1**a).

2. Systems Biology for Cancer Precision Medicine

2.1. Introduction to Systems Biology

Systems biology is an interdisciplinary science that combines biology, mathematical modeling, and computer simulation analysis to achieve a system-level understanding of complex biological phenomena (Figure 1b).[12–20] In systems biology, mathematical modeling of complex biomolecular regulatory networks is essential,[21] and diverse network control theories are being developed to move from understanding to controlling the dynamic behavior of living systems.[22,23] The biomolecular regulatory network models are constructed by integrating multiples types of omics data.[24,25] By performing dynamic simulations of a mathematical model of information flow through the network, hidden mechanisms controlling biological phenomena are revealed. With this new information, predictions regarding the phenotypic effects of perturbations on the network components can be generated and tested. In general, mathematical approaches to model biological networks can be classified according to the types of mathematical abstraction used to represent various cellular states of a system: either the system is simplified as discrete model with on or off states, or the system is represented with continuous model using differential equations or partial differential equations.[26–39]

2.2. Network Dynamics and Drug Resistance

Multiple signaling pathways form a complex signaling network within a cell. This signaling network not only delivers information of signals from upstream to downstream but also processes such information through complex network dynamics to dictate appropriate cellular responses.[40] Various circuit motifs in the signaling network are involved in this process with different motifs exhibiting distinct signal-modulating functions.[41] Positive feedback loops are a type of motif that amplifies signals and can generate ultrasensitive, all-or-none, bi-stable responses. Consequently, by creating bi-stable switches, positive feedback loops transform continuous graded signals into discrete signals. Negative feedback loops are another type of motif that can generate oscillating or cyclical behavior in various biological systems. A third type of motif, incoherent feedforward loops, can produce dose-dependent biphasic responses. A signaling network with these circuit motifs, which are common in biological networks, has complex input-output relationships.

Systems biological analysis can unravel the mechanisms producing such complex input-output dynamics. One example of a complex response in a biological network was observed for the response of the cell cycle regulatory protein Cyclin D.[42] When cultured colorectal cancer cells were stimulated with the extracellular ligand Wingless-related integration site

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Kwang-Hyun Cho is a professor in the Department of Bio and Brain Engineering at KAIST and a director of the Laboratory for Systems Biology and Bio-inspired Engineering. He started systems biology by his own idea of combining control engineering and biology from 1999.

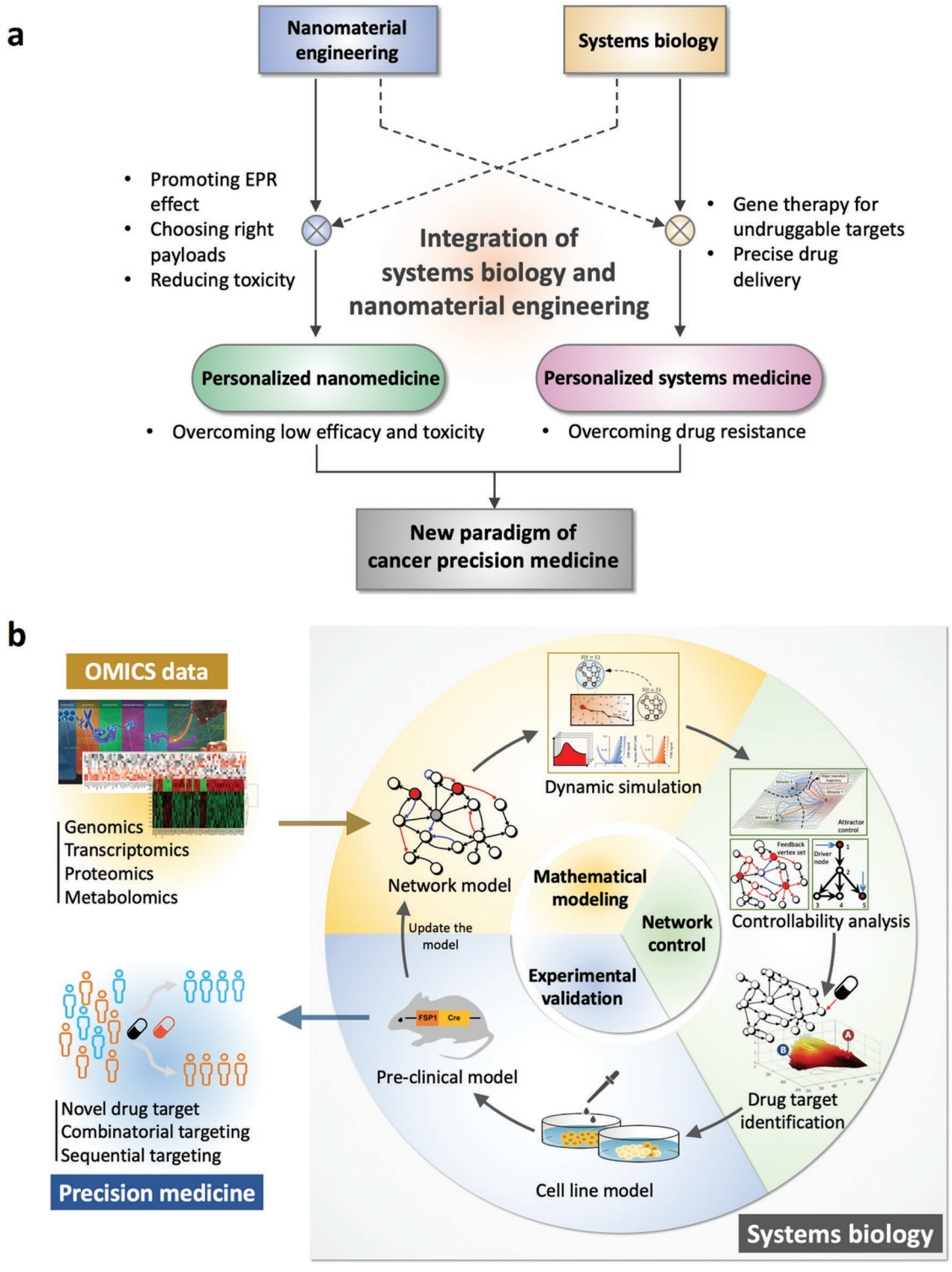

Figure 1. Integration of systems biology and nanomaterial engineering to achieve precision medicine. a) A schematic representation of integrating two fields. Systems biology and nanomaterial engineering can overcome the challenges of each field in achieving personalized nanomedicine and systems medicine for a new paradigm of cancer precision medicine. b) A schematic representation of the application of systems biology for precision medicine. Mathematical modeling of complex molecular regulatory networks, identification of molecular targets to control the dynamic behavior of complex networks, and experimental validation form the cyclic process of the systems biological approach. By integrating patient omic data in this process, systems biology can explore new drug targets and identify combinatorial or sequential drug targets for precision medicine.

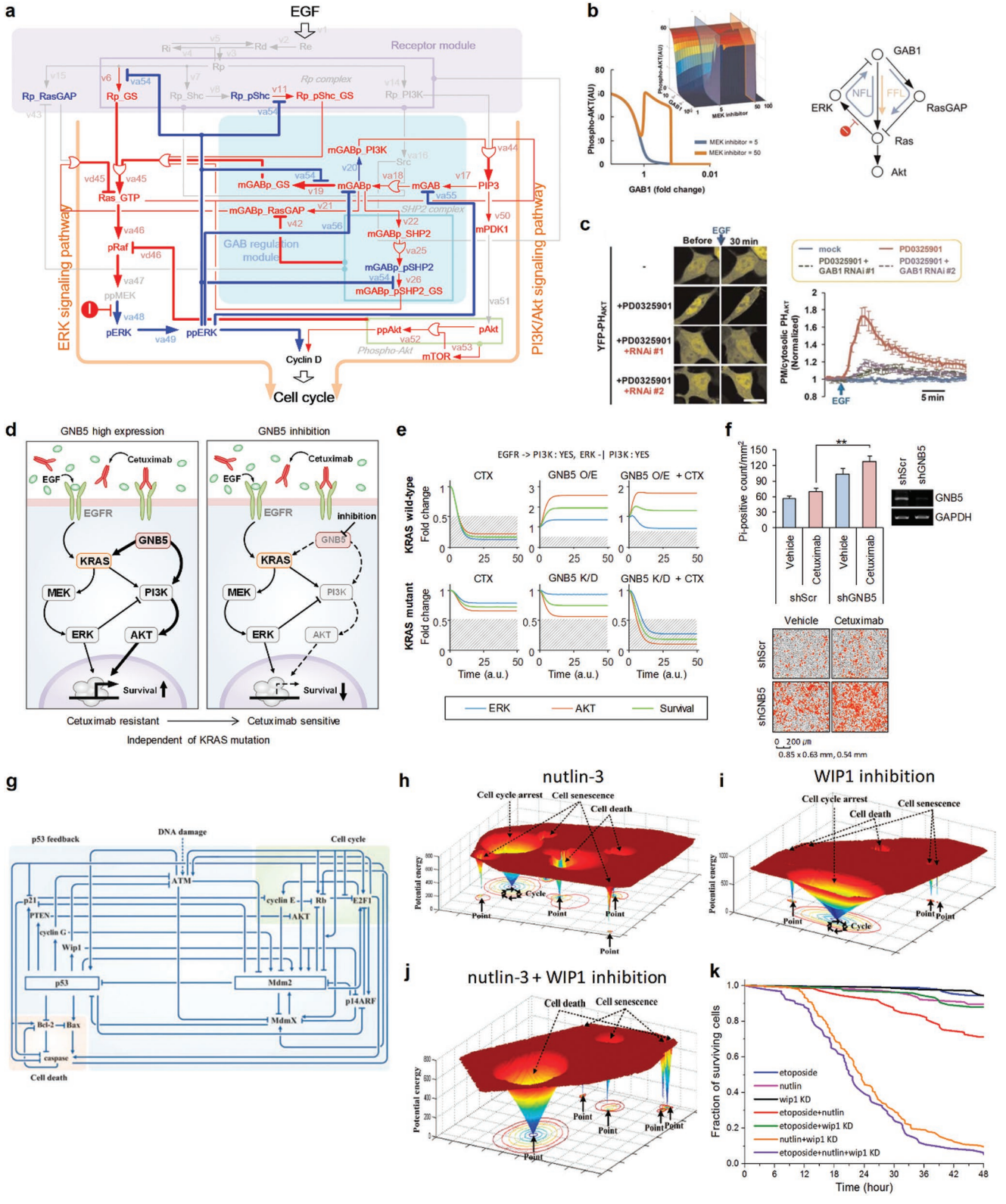

Figure 2. Identification of novel targets to overcome drug resistance in complex signaling networks using systems biology. a) Signal flux changes in a signaling network of the MAPK and PI3K-AKT pathways after MEK inhibition. The signal through the PI3K-AKT pathway was increased by MEK inhibition. Red or blue lines indicate increasing or decreasing paths of signal flux between nodes (representing biological molecules), respectively. b) Simulation of the network model predicted GAB1 as an effective combinatorial target to be inhibited with MEK inhibition. c) Experimental validation that resistance to MEK inhibitor was reduced by inhibition of GAB1 in HEK293 cells. a–c) Reproduced with permission.[44] Copyright 2012, Oxford

(WNT), the activity of Cyclin D depended not only on current WNT concentration but also on past history of exposure to WNT and the concentration of such previous exposures. This dependence on previous exposure for the present response is called hysteresis. This hysteretic response resulted from coupled positive and negative feedback loops in the WNT signaling network.[42] Another study observed that stimulating cultured neurons with different temporal gradients of brain-derived neurotrophic factor (BDNF) produced different cellular responses that resulted from interconnected positive and negative feedback loops.[43] By identifying such circuits within a larger complex network, systems biology can uncover the critical circuits to effectively target with new drugs, as well as the circuits that are involved in the responses to currently used drugs. Additionally, because systems biology reveals dynamic properties of networks, this approach can guide the kinetics of drug delivery.

Because complex dynamics confer drug resistance through mechanisms that emerge in a complicatedly intertwined signaling network, systems analysis of network dynamics based on mathematical modeling and computer simulation analysis is useful. The power of this approach is illustrated by the 2012 study from Won et al., who constructed a mathematical model for a network of two key pathways—the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinases (PI3K)-AKT pathways—involved in cancer cell survival, growth, and proliferation (**Figure 2**a).[44] Both pathways contain various feedback loops and are connected by crosstalk regulation. From the analysis of signal flux in this network after the simulation of an inhibitor for the MAPK kinase (MEK), and integration of the findings into the context of a larger cancer cell network, the authors determined that suppression of the MAPK pathway by an MEK inhibitor can cause compensatory activation of the PI3K-AKT pathway, resulting in drug resistance. Simulations were performed of the activities of the MAPK and PI3K-AKT pathways, represented by the amount of the active form of one of the proteins in each pathway, after MEK inhibition in networks representing diverse mutation conditions. The results of these simulations were similar to those obtained in experiments with cells having the same mutations and exposed to an MEK inhibitor, validating the predictions of the network analysis. Furthermore, the simulations with the networks representing mutation conditions predicted that the compensatory activation of PI3K-AKT pathway to an MEK inhibitor was high in cells with the B-rapidly accelerated fibrosarcoma (BRAF) mutation. Not only did these results suggest a combination therapy, but they also indicated the best candidates for this treatment, patients with BRAF mutations. Indeed, preclinical studies combining a PI3K inhibitor (LY294002) with an MEK inhibitor (PD0325901) produced a synergistic reduction in cell viability of two BRAF-mutant melanoma cell lines, SK-Mel-1 and SK-Mel-5.[44]

Beyond the MAPK and PI3K-AKT pathways, negative feedback loops from downstream to upstream within a pathway and crosstalk connections between various pathways maintain cellular homeostasis.[45] Such negative feedback loops form circuits that function as negative feedback amplifiers and can contribute to drug resistance.[46] Thus, drug inhibition of a protein in such circuits can relieve the negative feedback, activate upstream signaling in the targeted pathway, and subsequently induce reactivation of the targeted pathway or compensatory activation of bypass signaling pathways.[47,48] Although some mechanisms by which cells adapt to anticancer drugs are known, these adaptive responses involve dynamic reprogramming of the cell's network. For example, after treatment with a drug targeting one of the pathways, dynamic reprogramming of the cancer signaling network occurs. This reprogramming enables the cells to adapt to the presence of the drug and counteract its effect so that the cancer cells withstand the drug treatment.[49–52] To discover how network dynamics produce additional mechanisms of resistance and determine how the extensive reprogramming contributes to resistance, a systems biological approach is necessary.

3. Nanomaterial Engineering Can Overcome Challenges of Therapies Identified through Systems Biology

3.1. Combinatorial Therapy with Undruggable Novel Targets to Overcome Resistance

As indicated by the Won et al. study,[44] analysis of cellular network dynamics through mathematical modeling can discover drug combinations to overcome drug resistance. However, this approach can reveal not only known targets to be used in combination together but also novel targets for combinatorial therapy. Here, we describe representative studies covering various cancer-relevant signaling pathways with clinically used drugs for which patients show resistance.

Various MEK inhibitors are approved for cancer treatment. Using a network model of MAPK and PI3K signaling pathways, the 2012 study by Won et al. analyzed the resistance mechanism of MEK inhibitor (PD0325901) and determined that resistance

University Press. d) A network model for cetuximab resistance involving GNB5. e) Simulation of the network model for cells with wild-type KRAS or mutant KRAS predicted GNB5 as an effective combinatorial target to be inhibited with an EGFR inhibitor (cetuximab) in the context of mutant KRAS. Gray hatched areas indicate responses below the sensitivity threshold for survival, ERK activity, and AKT activity. CTX, cetuximab; GNB5 O/E, GNB5 overexpression; GNB5 K/D, GNB5 knockdown. f) Experimental validation of the effect of reducing GNB5 by shRNA on enhancing cell death assay in the colorectal cancer cell line HCT116 exposed to cetuximab. Top shows quantified results and bottom shows representative images taken 72 h after cetuximab treatment. d–f) Reproduced with permission.[53] Copyright 2019, Federation of European Biochemical Societies, published by John Wiley and Sons. g) p53 network model with the cell death module highlighted in pink and the cell cycle module highlighted in green. This model was used for attractor landscape analysis. h–j) Attractor landscape results following nutlin-3 treatment to inhibit the link between MDM2 and p53, inhibition of the phosphatase WIP1, and inhibition of WIP1 and exposure to nutlin-3. k) Experimental validation of the synergistic effect of WIP1 knockdown with siRNA and nutlin-3 in cells (orange) and the synergistic effect of this combination with a DNA-damaging agent (etoposide, purple). g–k) Reproduced with permission.[58] Copyright 2012, American Association for the Advancement of Science.

to MEK inhibition occurs from bypassing the blocked MAPK pathway and activating the PI3K pathway (Figure 2a,b).[44] The bypass involved reactivation of upstream parts of the pathway, thus the authors analyzed the effect of combining an MEK inhibitor with reduced function of other proteins that are upstream of the MAPK and PI3K pathways. These included the 3-phosphoinositide-dependent protein kinase-1 (PDK1), the adaptor proteins Son of Sevenless (SOS), and the growth factor receptor-bound protein 2-associated-binding protein 1 (GAB1). Overall, their results showed that GAB1 is the critical mediator that can induce signaling to activate PI3K after MEK inhibition. In preclinical experiments, GAB1 was verified as a promising candidate to overcome drug resistance of MEK inhibitor by reducing GAB1 abundance through RNA interference (RNAi) (Figure 2c). Unfortunately, reducing GAB1 abundance or interfering with GAB1 function is not easy to be achieved through traditional drugs and delivery mechanisms.

Cetuximab is an antibody that targets epidermal growth factor receptor (EGFR), which activates multiple signaling pathways including the MAPK pathway. Park et al. found a new target to overcome resistance of cetuximab using a systems biological approach (Figure 2d).[53] They identified five potential targets to inhibit that were predicted to prevent resistance to cetuximab. Kirsten rat sarcoma viral oncogene homolog (KRAS) is a guanosine triphosphate (GTP)-binding signal-transducing protein in the network and is activated by EGFR. Activating mutations in KRAS represent a mechanism of resistance to EGFR inhibitors.[54–56] However, other mechanisms of resistance exist, because cells with wild-type KRAS can be resistant to cetuximab. Importantly, reducing the abundance of the candidate target, the G protein subunit beta 5 (GNB5), induced cetuximab sensitivity in cells with wild-type or mutant KRAS (Figure 2e). The predicted results were validated using experiments with colorectal cancer cells and they confirmed that the combination of cetuximab and reduction in GNB5 abundance enhanced the efficacy of either alone (Figure 2f). Not only did this study identify a novel target, but the target identified is a protein that is not typically included in the EGFR signaling network. Thus, the integration of omics data, network modeling, and computational analysis revealed fundamental new insight into the biology of these cancer cells as well as identified potential combinatorial drug targets to overcome drug resistance. Like GAB1, GNB5 is not an enzyme and is difficult to be functionally inhibited through conventional approaches.

Sorafenib is a multispecific kinase inhibitor that is the first line treatment in hepatocellular carcinoma (HCC). However, its therapeutic effect on overall survival of patients is highly variable. A 2017 study by Won et al. found not only a biomarker that can predict the response to sorafenib but also a novel drug target for combination therapy with sorafenib using a systems biological approach.[57] To unravel the hidden mechanism of drug resistance and predict strategies to enhance the efficacy of sorafenib, the authors performed gene set enrichment analysis (GSEA) of HCC cell lines exposed to sorafenib, then applied network dynamics analysis using mathematical modeling. The GSEA indicated that sorafenib causes proteotoxic stress and activates apoptosis pathways. The network simulation results predicted that combinatorial treatment of sorafenib and a protein disulfide isomerase (PDI) inhibitor, such as PACMA 31, would synergistically reduce cell viability, which was validated through experiments with cultured cells and with a mouse xenograft model. By integrating the system biology results with information related to survival based on *PDI* expression, the study shows how system biology aids in predicting which patients might benefit the most from sorafenib (those with low *PDI* expression) and which would require the combination of sorafenib and a PDI inhibitor (those with high *PDI* expression) for effective treatment. In this case, simultaneous administration is required to obtain the predicted synergism which is challenging to be achieved in vivo due to heterogeneous physicochemical characteristics of drugs.

Kinases and kinase-mediated pathways are not the only targets for cancer therapy. The DNA damage and stress response pathway mediated by the transcription factor p53 is also a critical pathway contributing to cancer cell survival. The cellular response to p53 activity depends on the dynamics of p53 activation, such as an oscillating activation and a sustained activation. Responses include cell cycle arrest or cell death, which are states that correspond to attractors in models. Choi et al. constructed a p53 regulatory network model and analyzed its regulatory dynamics with in silico perturbation experiments that mimic the effect of different drugs under various conditions (Figure 2g).[58] Using attractor landscape analysis, the authors identified a combinatorial strategy for reducing cell viability using nutlin-3, which increases p53 abundance by inhibiting the interaction between the E3 ubiquitin ligase mouse double minute 2 homolog (MDM2) and p53, and inhibition of a wild-type p53-induced gene (WIP1) (Figure 2h–j). The in silico experiments predicted that cells with DNA damage, such as that caused by radiation treatments or traditional genotoxic chemotherapeutics, would undergo apoptosis in response to the combination of nutlin-3 and WIP1 inhibition. This prediction was validated using single-cell experiments (Figure 2k). Here, the challenge is achieving controlled simultaneous delivery of genotoxic agents, nutlin-3, and the WIP1 inhibitor or a reagent that reduced *WIP1* expression.

In a second study that used the same approach of attractor landscape analysis, Choi et al. analyzed panels of cancer cells to characterize different cancer subtypes.[59] They used large-scale cancer cell genomic data available from Cancer Cell Line Encyclopedia (CCLE) to construct different molecular networks representing distinct characteristics of genetic variations of diverse cancer subtypes. They performed in silico perturbation analysis of drug responses in each molecular network to identify synergistic combinations of drugs. The predicted results were then compared to results of experiments with various cancer cell lines, including lung, breast, bone, skin, kidney and ovary cancers. The in silico predictions correlated with the experimental results. This strategy can be applied to any molecular network to identify an optimal drug target for precision medicine, the causes of drug resistance, and potential combinatorial drug targets to overcome resistance.

Although systems biological studies have identified novel drug targets and combinatorial therapy to overcome drug resistance, there are still remaining challenges to be solved. Many of the identified novel targets are considered undruggable, because they lack enzymatic activity or specific inhibition is difficult to be achieved. Thus, one option for inhibiting their

function is safe and effective gene therapy. Additionally, the combination therapies require a specific mechanism to deliver the drugs simultaneously to the tumor cells, which needs a specialized delivery system. Nanomaterial engineering can overcome these limitations and challenges by encapsulating gene-targeted therapies and providing targeted delivery systems with precise release kinetics.

3.2. Nucleic Acids as Therapeutics Using Nanocarriers

For the novel targets that are considered undruggable through conventional small-molecule or antibody-based methods, gene therapy using gene editing and RNAi are options for reducing the function of the newly identified targets. These forms of regulation rely on the delivery of various forms of nucleic acids into the appropriate cells. For proper delivery, the nucleic acids introduced into the body must be stably transported to the target cells. However, the body has multiple mechanisms to eliminate nucleic acids that are not inside cells. Various endogenous nucleases rapidly degrade long DNA or RNA molecules, and small RNAs, such as small interfering RNAs (siRNAs), accumulate rapidly in the kidney and are then excreted in the urine.[60,61] Moreover, even if the nucleic acid arrived at the target cell, the negatively charged surface of the nucleic acid prevents its spontaneous permeation across the cell membrane. For nucleic acids that are internalized into the cell, they need to escape from endosomes and lysosomes without being degraded and either function in the cytoplasm to interfere with translation or transport into the nucleus to interfere with gene expression. For these reasons, delivery of nucleic acid has a low efficacy.[62]

To improve the efficacy, the nucleic acid can be delivered in a viral vector or transported in nonviral vector carrier like a nanocarrier. For viral vectors, the viruses vary depending on which cells to target or what nucleic acids are loaded. Adenovirus can be loaded with a larger size of nucleic acids than other viruses, but this viral vector usually causes strong immune responses.[63] Nucleic acid delivered by adenoviral vectors is not integrated into the cell's genome, thus the nucleic acid is degraded gradually in the cytoplasm. In contrast, nucleic acid delivered by retrovirus or lentivirus vectors becomes integrated into the genome of the target cell where it can be stably and continuously expressed, depending on the location of integration. However, the length of nucleic acids that can be incorporated into retroviruses or lentiviruses is limited, and integration into the patient's genome can cause undesired insertional mutagenesis.

Unlike viral vectors, a nanocarrier is a safe and effective delivery platform for administering various forms of therapeutic agents, including nucleic acids (**Figure 3**a). Moreover, nanocarriers are easily modified to avoid clearance by mononuclear phagocytes, enhance the delivery to the target cell, transport across cell membranes, and escape from endosomes and lysosomes.[62,64–67] Furthermore, drug or gene therapy mediated by nanocarriers has the potential to achieve improved responses compared with those delivered without using nanocarriers (Figure 3b).Thus, many researchers have been trying gene therapy using nonviral vectors instead of viral vectors.

For instance, onpattro is the first Food and Drug Administration (FDA)-approved RNAi-based drug. Onpattro is used to treat peripheral neuropathy due to hereditary amyloid transthyretin (ATTR) amyloidosis, which is a rare disease caused by a mutation in the transthyretin gene (*TTR*). The RNAi molecules are delivered in lipid-based nanocarriers, which are transferred into hepatocytes where the RNAi suppresses *TTR* mRNA to decrease amyloid deposits and block neuropathy.[68]

Other nanocarrier compositions, such as those with positively charged lipids or polymers, target specific sites in the body.[62,69,70] For instance, RNAi molecules delivered by lipid-like 7C1 polymer result in specifically targeting endothelial cells.[71,72] The 7C1 polymer can be synthesized by conjugating C15 epoxide-terminated lipids with the low molecular weight polyamine, polyetherimide (PEI) 600 polymer (**Figure 4**a–c). This polymer is then degraded in certain environments, but it is not taken up by hepatocyes and immune cells. Such a transfer system carrying siRNA targeting a critical growth factor receptor or a ligand for endothelial cells effectively reduced tumor volume and metastasis in a mouse model of lung carcinoma (Figure 4d–g).

DNA in the form of a circular plasmid or messenger RNA (mRNA), which are longer than RNAi molecules, can also be loaded into nanocarriers to effectively express a protein in a target cell.[73–76] Moffett et al. and Smith et al. each developed a nanocarrier that specifically targets the T cells of patients and delivers mRNA or a DNA plasmid into the T cells to transform them into chimeric antigen receptor (CAR) T cells in situ or ex vivo.[77,78] In this nanocarrier, mRNA or a DNA plasmid encoding the CAR is condensed by incubation with poly(beta-amino ester) (PBAE) 447 polymer and a polyglutamic acid-antibody recognizing cluster of differentiation 3 (CD3), a protein found on the surface of T cells. Through the CD3-specific antibody, the nanocarrier interacts with T cells. For nanocarriers with the DNA plasmid, additional loading of a peptide with microtubule-associated sequences and nuclear localization signal (MTAS-NLS) helps penetration of the plasmid into the nucleus.

The targeted genome editing tool, CRISPR-Cas9 has been combined with nanocarriers for therapeutic use.[79–84] Studies have tested using either nucleic acid or protein for the Cas9 component. The challenge of using Cas9-encoding nucleic acids is that these tend to produce a greater immune response than that observed with Cas9 protein.[85,86] However, Cas9 protein is not effectively taken up by cells in an animal model, because it is unstable in circulation and is negatively charged. Loading single guide RNA (sgRNA) and Cas9-encoding nucleic acids into nanocarriers enabled a highly efficient genome editing in vivo and reduced the amount of TTR in circulation by ≈97% in 12 months in a mouse model.[83] Sun et al. developed a CRISPR-Cas9 delivery platform based on DNA nanoclews, which are nanosized delivery vehicles consisting of DNA that is coiled into compact structures resembling balls of yarn. Self-assembled single-strand DNA was combined with partially complementary sequences to the sgRNA and Cas9 protein/sgRNA complexes. The Cas9/sgRNA complex-loaded DNA nanoclew was encapsulated in a cationic polymer, which was then coated with PEI to maximize cellular uptake and endosomal escape.[87] Chen et al. developed liposome-templated hydrogel

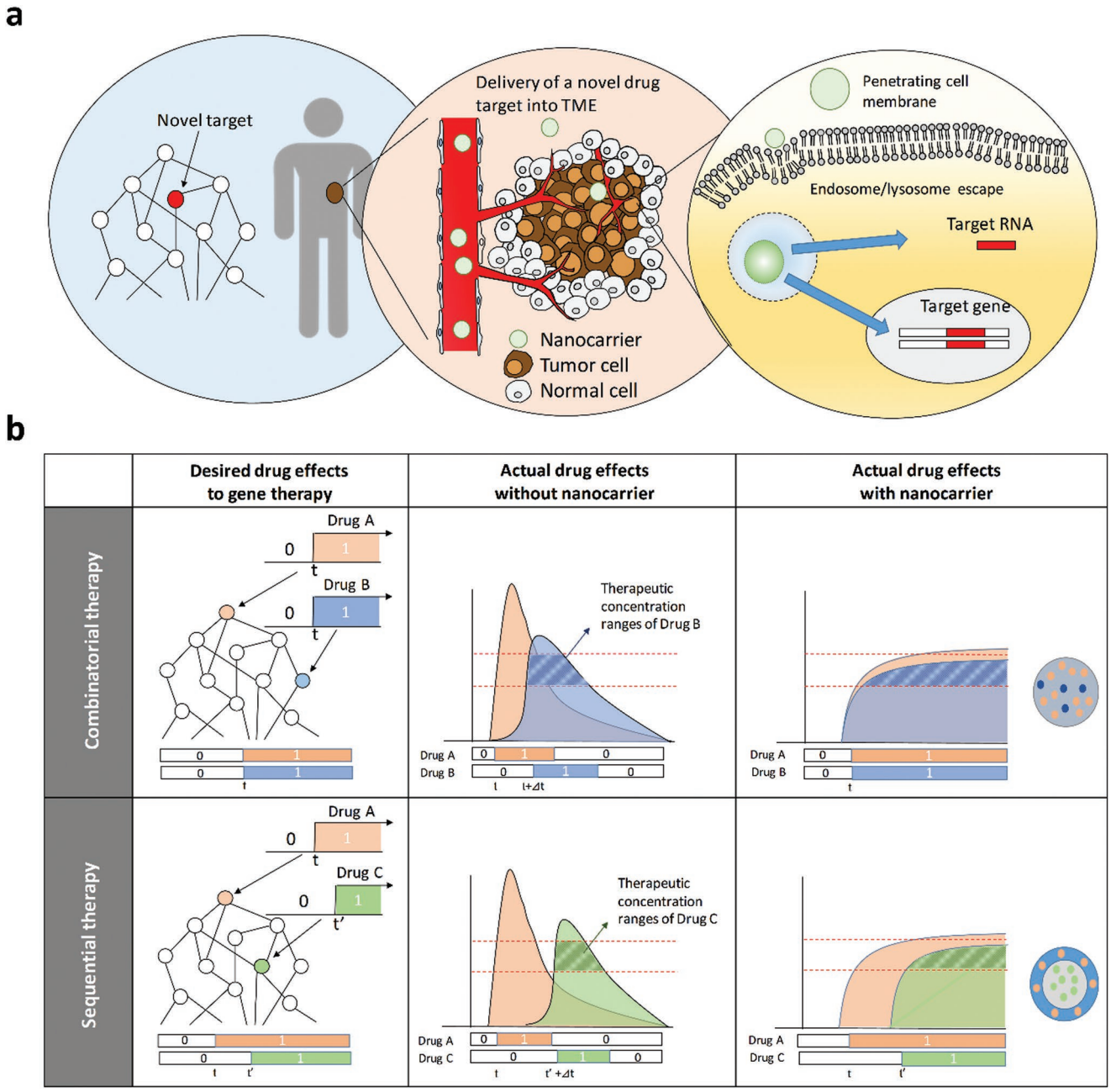

Figure 3. Schematic representation for nanomedicine and its advantages over treatment without using nanocarriers for drug delivery. a) Nanocarriers can effectively deliver therapeutic agents to tumors, including therapeutic agents such as nucleic acids that modify gene expression. In the first panel, systems biology identifies a novel, but difficult to inhibit, target. In the middle panel, the nanocarrier transports a nucleic acid-based therapy to the tumor cells to inhibit the production of the identified target. In the last panel, the tumor cells endocytose the nanocarrier, which releases its payload that inhibits expression of the target. b) The advantages of drug delivery by nanocarriers for simultaneous and sequential combination therapy. Delivery of the therapeutic agents with nanocarriers achieves more effective simultaneous (upper row) or sequential combination therapy (lower row) than delivery without using a nanocarrier. The last panel in each row shows a diagram of the nanocarrier with the two drugs in the same colors as the responses are shown in the graphs and the respective targets are shown in the networks in the first panels. t, starting time of Drug A or B treatment; t': starting time of Drug C treatment; Δt, undesired delay time.

nanocarriers with a core composed of a PEI hydrogel that can be loaded with Cas9 protein and sgRNA.[88] To maximize penetration across the cell membrane, this core is surrounded by a liposome shell of dioleoyl-3-trimethylammonium propane (DOTAP). Additionally, DOTAP liposomes with the hydrogel core more effectively encapsulate the Cas9 protein than do DOTAP liposomes without the hydrogel: 62.8% incorporation versus 6.3%.

Peptide nucleic acids (PNA) are synthetic DNA analogs with a neutral charge that can bind to specific sequences of DNA

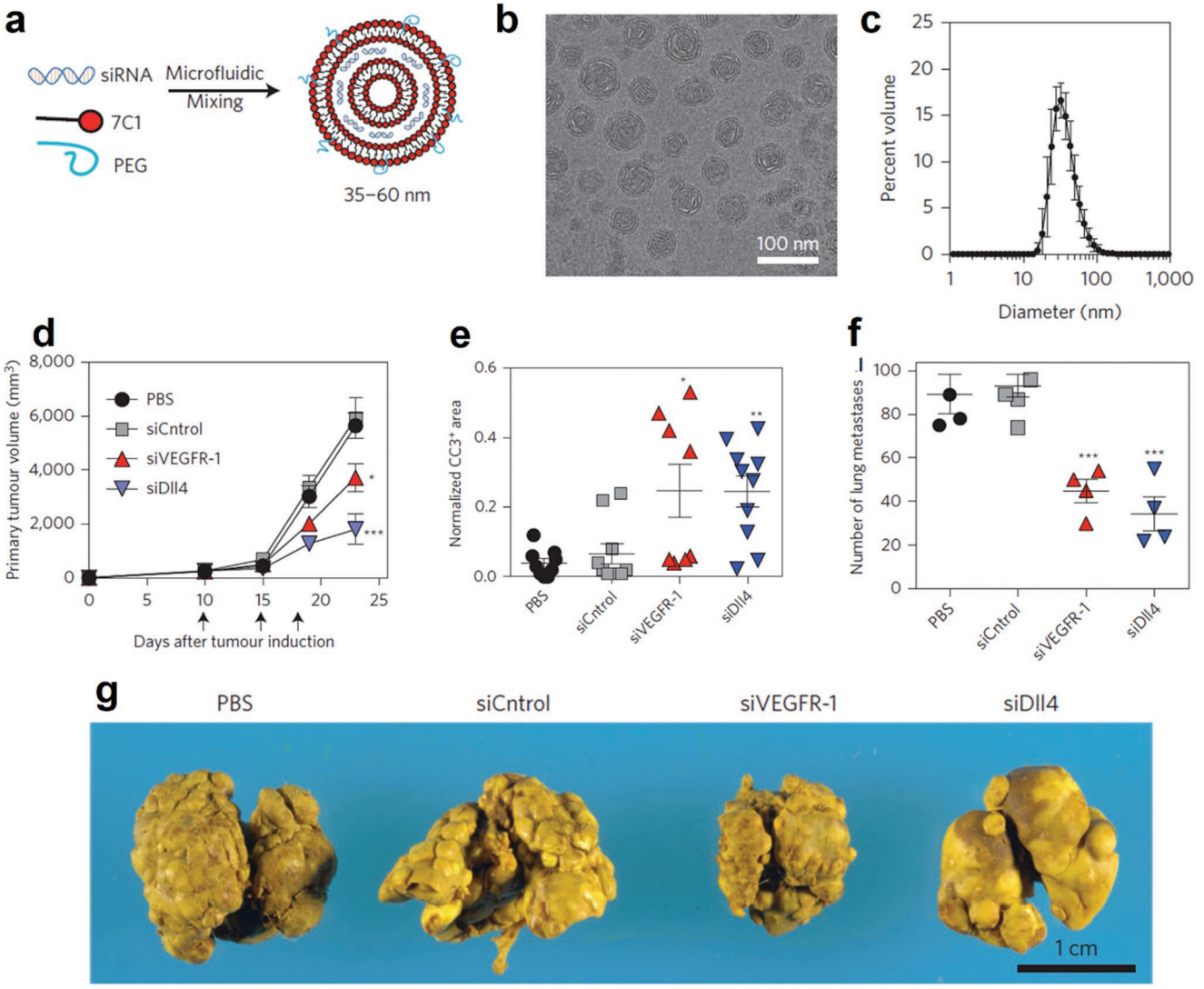

Figure 4. Delivery of siRNA to inhibit gene expression using nanocarriers. a) Diagram of the formulation of the 7C1 nanocarrier with siRNA. Poly(ethylene glycol) (PEG) is incorporated on the exterior layer. b) Cryo-TEM image of the 7C1-based nanocarrier. c) The size of 7C1 based nanocarrier measured by dynamic light scattering. d) Reduction in tumor volume, e) induction of apoptosis, and f) reduction in lung metastasis using siRNA targeting VEGFR or DLL4 delivered by 7C1-based nanocarriers in a mouse tumor model. g) Representative images of mouse lungs with metastatic lesions from mice administered the indicated treatments. a–g) Reproduced with permission.[71] Copyright 2014, Springer Nature.

or RNA. Moreover, PNA are metabolically stable due to their unnatural backbone structure, which is not degraded by nucleases or proteases. These synthetic molecules can be loaded into nanocarriers for gene editing. For instance, PNA molecules can form a triplex structure PNA-DNA-PNA with a donor DNA molecule and this unique structure induces an endogenous DNA repair process, during which the donor DNA is inserted into cell's genome.[89] Ricciardi et al. developed a poly(lactic-*co*-glycolic acid) (PLGA) nanoparticle carrying PNA and donor DNA encoding normal beta-globin that successfully treated beta-thalassemia in a mouse embryo by replacing the mutated beta-globin-encoding gene with that of the normal donor DNA.[90]

These studies show that nanocarriers can be used to establish safe and effective gene-targeted therapy. Thus, the application of nanomaterials engineering makes it possible to inhibit the undruggable targets that are identified through systems biological approaches. Additionally, nanomaterials engineering presents a multifunctional and customizable approach to delivering individual therapeutics or combinations of therapeutics to specific cells.

3.3. Combinatorial Therapy Using Nanocarriers

Controlling a single target is insufficient in many cases for achieving a desired cellular outcome due to the complex dynamics of biomolecular networks that can induce drug resistance. To overcome drug resistance, many systems biological studies suggest combinatorial therapies and even specific sequences of treatment. In addition, cancer cells have multiple mutations that vary not only from patient to patient even for cancers of the same tissue but also vary within a

patient due to tumor heterogeneity. Hence, to overcome the complexity of cancer, targeting multiple molecules is necessary (Figure 3b, first row, first column). When treating a patient with multiple drugs targeting the same cell or population of cells simultaneously, the therapeutic agents must reach the same place at the same time to maximize the efficacy. However, the physicochemical properties of drugs are often quite different, resulting in different timing for the drugs to reach the target cells and different periods of activity (Figure 3b, first row, middle column). Nanocarriers can be engineered to carry more than two drugs and release them at the same time to sustain the concentrations of both drugs (Figure 3b, first row, last column).[91,92]

Several nanocarriers loaded with multiple therapeutic agents have been tested in clinical trials with some receiving FDA approval for treating cancer.[93–96] For instance, administration of the pair of conventional cytotoxic chemotherapeutic agents, daunorubicin and cytarabine, in combination has been used for many years to treat acute myeloid leukemia. However, differences in their pharmacokinetics and pharmacodynamics make it difficult to achieve the optimal molar ratio to maximize their synergistic effect in vivo. The FDA-approved drug CPX-351 contains these two drugs in a liposomal nanocarrier and overcomes this limitation.[97] CPX-351 contains cytarabine and daunorubicin at a 5:1 molar ratio. This molar ratio of the drugs applied directly to culture medium has the highest synergy with the lowest antagonism in tests of 15 types of tumor cell lines.[98] Among liposomes loaded with cytarabine and daunorubicin in various molar ratios, the 5:1 molar ratio shows the greatest therapeutic efficacy in animal models,[98] thus this is the ratio used in CPX-351.

Nanocarriers can contain not only multiple chemotherapeutic agents or small molecule drugs but also other forms of therapeutic agents, like multiple nucleic acids, multiple proteins, or various combinations of nucleic acids, proteins, and conventional drugs. Indeed, administration of both RNAi and chemotherapeutics by a single nanocarrier might overcome multidrug resistance and enhance anticancer effects.[99–101] Xiong et al. developed a nanocarrier containing an siRNA and doxorubicin.[102] They encapsulated an siRNA targeting the gene encoding multidrug resistance 1 (*MDR1*) using poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL), a block copolymer. Encapsulation occurred through an electrostatic interaction between the siRNA and the amine groups of PCL. Doxorubicin was chemically conjugated to the polymers. This delivery system is stable when administered to mice and delivers both the siRNA and the conventional chemotherapeutic agent into tumor tissue in a mouse model of cancer.

In addition, multiple nucleic acids controlling different targets have been loaded into nanocarriers.[103–105] Lee et al. co-polymerized two siRNAs, loaded this dual-gene targeting siRNA polymer (Dual-poly-siRNA) into thiolate glycol chitosan (tGC) nanoparticles, and successfully silenced the two target genes (**Figure 5**a).[106] Because the siRNA polymers are based on thiol bonds that are unstable inside cells, the active siRNA molecules were released upon reaching the cytoplasm. They used siRNAs for vascular endothelial growth factor (VEGF) and the cell death-inhibiting protein B-cell lymphoma 2 (Bcl-2). The nanocarrier with the combination was more effective in inhibiting cancer growth in animal models than nanocarriers with each of the siRNAs delivered separately (Figure 5b–d). Another example of nanocarriers delivering multiple nucleic acids involves the encapsulation of an siRNA to reduce the abundance of an oncogene product and copies of an endogenous regulatory short RNA called a microRNA (miRNA or miR) that functions as a tumor suppressor. Xue et al. generated 7C1 polymer nanocarriers loaded with miR-34a and siRNA targeting *KRAS* for testing in animal models of lung adenocarcinoma.[107] The encapsulated miR-34a is a p53-regulated tumor suppressor that inhibits the expression of multiple target genes, thereby blocking the tumor growth.[108] *KRAS* is an oncogene particularly relevant in adenocarcinoma,[109] and knockdown of this gene induces apoptosis of cancer cells and blocks tumor growth.[110] Together, these studies demonstrate the possibility of using nanocarriers to deliver various types of therapeutic agents to the cancer cells simultaneously.

These studies show that nanocarriers can be generated to concurrently release heterogeneous payloads. Therefore, simultaneous combinatorial therapy to overcome drug resistance can be achieved by integrating nanomaterial engineering and systems biology.

4. Systems Biology Can Overcome the Challenges of Personalized Nanomedicine

4.1. Low Efficacy of Nanomedicine

Most nanomedicine fails to receive approval for clinical use due to low efficacy. Although 94% of nanodrugs pass phase 1 clinical trials, only 48% and 14% of nanodrugs show positive outcomes during phase 2 and phase 3 clinical trials, respectively.[111] Most negative outcomes are due to immunogenicity and insufficient drug efficacy, which is usually caused by failure in drug delivery to tumor.[111] In addition, a fundamental problem in successfully delivering nanocarriers to specific tumor sites in human patients is a lack of understanding of the EPR effect, which is observed in preclinical mouse models.[10,112–115] In animal models of cancer, the EPR effect is thought to result from the tumor's induction of immature blood vessel formation, which causes nanoparticles to be accumulated in regions where the tumor is highly vascularized with immature vessels and where the vessels are more permeable.[11,116] However, in clinical trials with patients, numerous nanocarriers fail because of very low (less than 1%) accumulation in tumors.[117] In addition, like in conventional drug therapy, heterogeneity in responsiveness of different subsets of patients limits nanocarrier-delivered drug efficacy.

Personalized nanomedicine is likely the future of nanomedicine, because this approach will take into account the heterogeneous characteristics of patients and, for cancer patients, the heterogeneity of cancer cells within a patient.[8–10] In the following two sections, we review how systems biology can contribute to realizing personalized nanomedicine by promoting EPR and aiding in target selection, and identifying treatment paradigms.

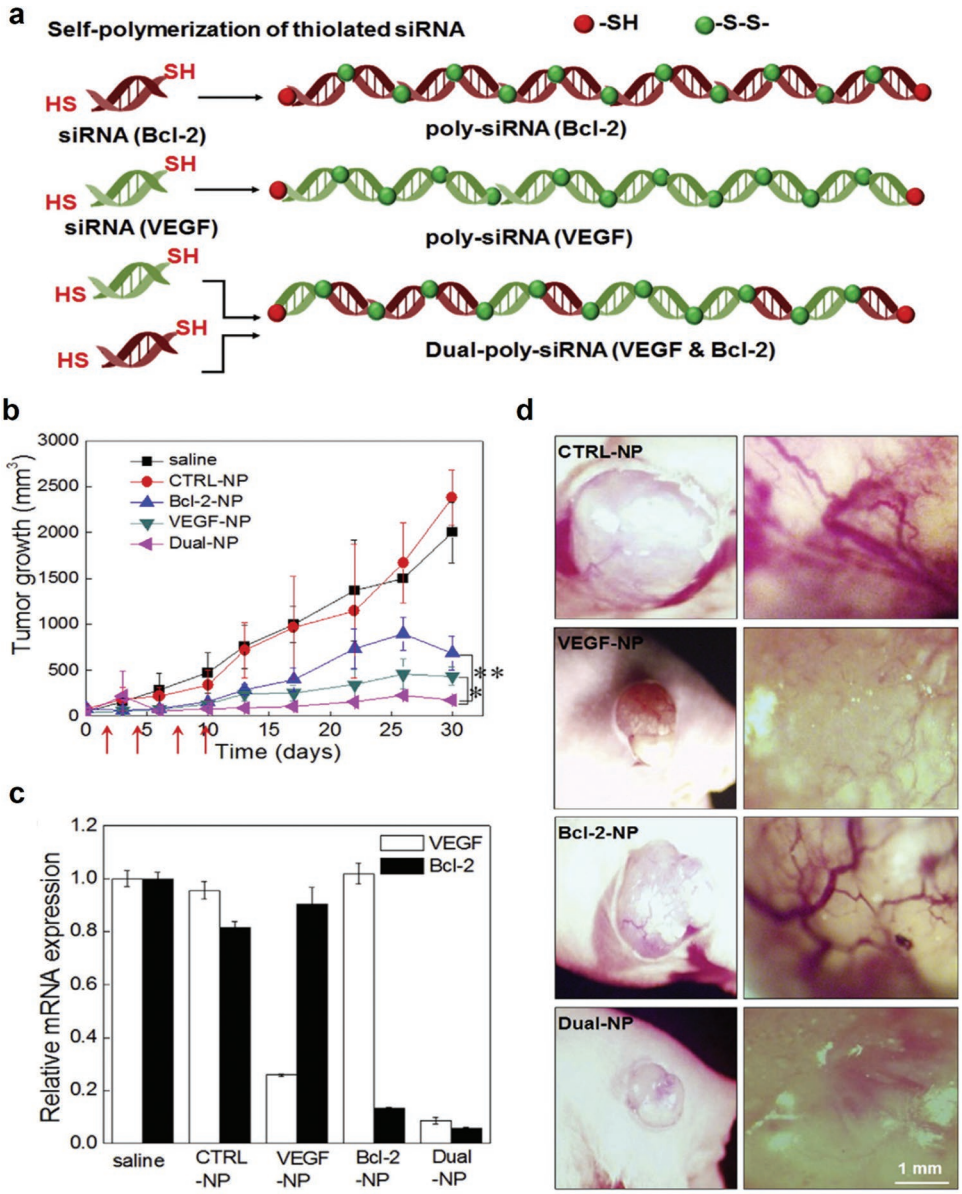

Figure 5. Combination of drugs delivery by nanocarriers. a) Schematic illustration of self-polymerizing individual siRNAs (poly-siRNA) and dual-poly-siRNA containing siRNA against two targets. These siRNAs were incorporated into nanoparticles (NPs). b–d) Dual-poly-siRNA delivered by nanoparticle (Dual-NP) is more effective than either single poly siRNA when tested in a mouse tumor model: b) Tumor growth was monitored. Nanoparticles were administered on the days marked by arrows. c) Quantification of VEGF and Bcl-2 mRNA from tumor tissue, and d) macroscopic images showing the effect of the nanoparticles on tumor blood vessels. a–d) Reproduced with permission.[106] Copyright 2015, Elsevier.

4.2. Using Systems Biology to Promote EPR of Nanomedicine

Strategies attempted to overcome the heterogeneous EPR observed among cancer patients include vessel promotion, disruption, permeabilization, and normalization. In particular, many studies have been conducted to promote EPR by inhibiting angiogenesis to let immature vessels mature, thereby normalizing the vessels.[11,116,118–120] Although intuitively vessel normalization may be expected to reduce the delivery nanocarriers into tumors by reducing vessel permeability and density, normalized vessels have increased permeability for nanoparticles compared to the permeability of immature vessels.[121–125]

The major signaling pathway for angiogenesis involves vascular endothelial growth factor A (VEGFA), which activates its receptor vascular endothelial growth factor receptor 2 (VEGFR2). Inhibiting the VEGFA/VEGFR2 pathway has been studied as a method to normalize the vessels of cancer patients.[126] Jiang et al. discovered that inhibiting the activity of VEGFR2 enabled vessel normalization, which enhanced the accumulation of a nanoparticle in the tumor (**Figure 6**a–c).[127] Some patients had intrinsic resistance to the VEGFR2 inhibitor, and others showed an initial positive outcome and then developed adaptive resistance to the inhibitor.[128,129] To effectively and persistently normalize vessels in all patients, understanding

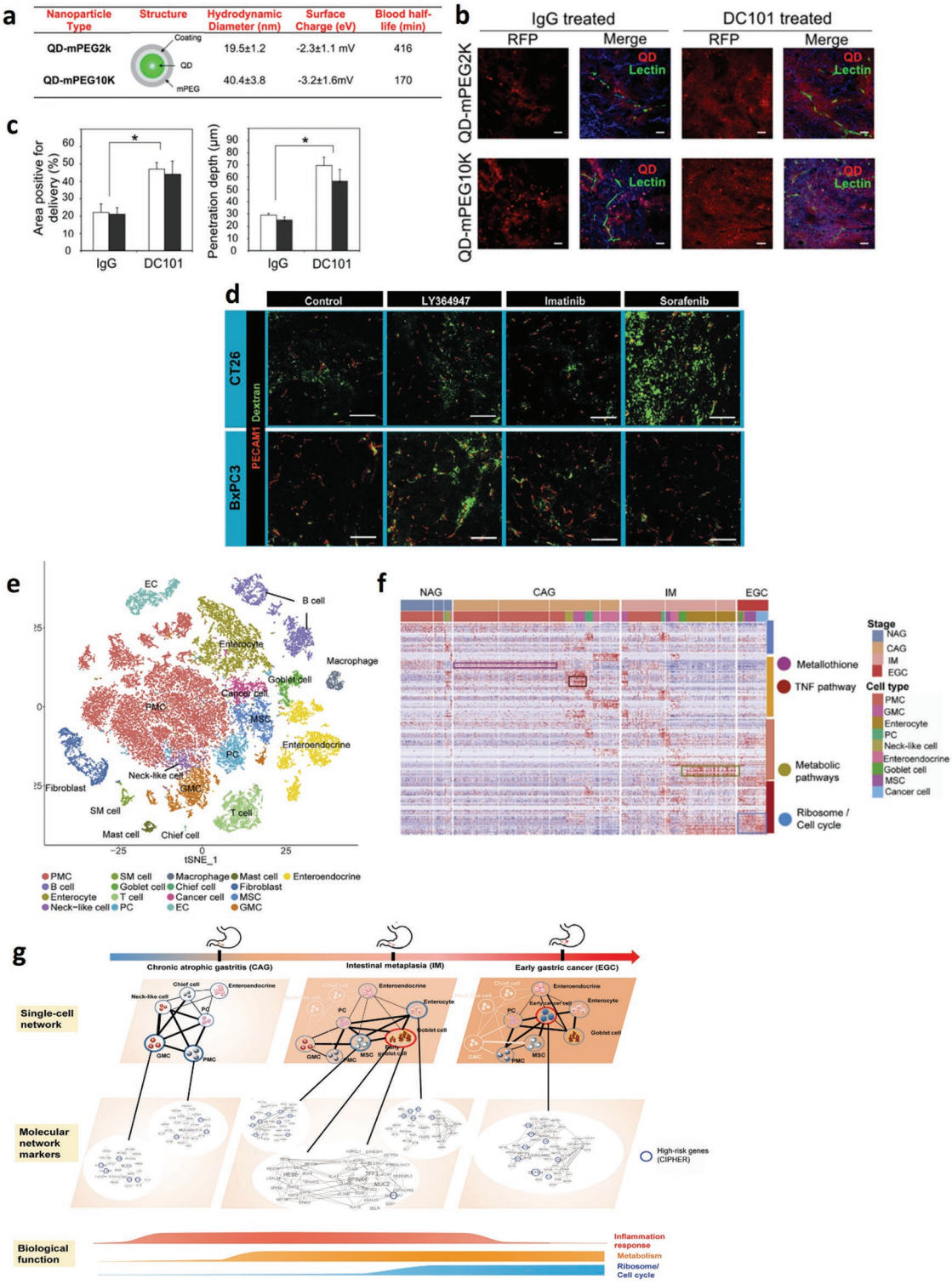

Figure 6. Using systems biology to promote EPR by providing in-depth information about the dynamics of the TME. a–c) Administration of a VEGF inhibitor (DC-101) to normalize blood vessels significantly enhances nanoparticle delivery in a mouse tumor model. a) Characteristics of two quantum dot-encapsulating nanoparticles. The main difference is the size of the particles. b,c) Pretreatment of the animals with DC-101 increases penetration of each nanoparticle into tumor sites. Red signals indicate the quantum dot nanoparticles and blood vessels are marked with a lectin (green). a–c) Reproduced with permission.[127] Copyright 2015, American Chemical Society. d) The effect of pericyte coverage of vessels and various kinase inhibitors on the penetration of nanoparticles containing 2 MDa dextran (green) into tumor tissue in mice xenograft models. CT26 xenografts have low pericyte coverage; BxPC3 xenografts have high pericyte coverage. Penetrance of nanoparticles into the tumor is greater in CT26 tumors and enhanced by pretreatment of the animals with the VEGF inhibitor sorafenib. Blood vessels are marked by PECAM1 staining (red). Nanoparticles are indicated with red. Imatinib: platelet-derived growth factor-B (PDGF-B)

the mechanisms of both intrinsic and adaptive resistance to such VEGFA/VEGFR2 pathway inhibitors is crucial.

Although the VEGFA/VEGFR2 pathway is important for angiogenesis, this process is regulated by multiple signaling pathways, all of which are complex and include various positive and negative feedbacks.[130–132] These complex circuits can generate complex network dynamics and drug resistance. In addition, crosstalks among the signaling pathways related to angiogenesis, such as pro-angiogenic signaling downstream of factors fibroblast growth factor 2 (FGF2) and phosphatidylinositol-glycan biosynthesis class F protein (PIGF),[133,134] can cause resistance to agents that stimulate vessel normalization. Consequently, systems biological approaches are required to identify the appropriate approaches to overcome these resistance mechanisms.

Various studies have applied system biology to investigate angiogenesis and identify novel targets for vessel normalization or biomarkers of responsive patients for vessel normalization. Abhinand et al. integrated 26 599 articles related to VEGFA and VEGFR2, and constructed VEGFA/VEGFR2 signaling network.[135] From the network, authors identified how the VEGFA/VEGFR2 signaling cascade regulates multiple downstream modules: the AKT-mammalian target of rapamycin (mTOR), c-Jun N-terminal kinase (JNK), phospholipase C (PLC)-protein kinase C (PKC), and nuclear factor kappa B (NFκB) modules. In addition, other groups constructed gene regulatory or protein-protein interaction (PPI) networks from whole genome or proteome information from patients.[136–140] For instance, Glass et al. suggested identifying biomarkers based on links in the gene regulatory network to stratify patients into responsive and resistant subtypes to vessel normalization.[141] From that, the authors identified that preventing interactions between aryl hydrocarbon receptor nuclear translocator (ARNT) and aryl hydrocarbon receptor (AHR) or hypoxia-inducible factor 1-alpha (HIF1a) represses angiogenesis. Thus, these studies show that, by constructing and analyzing signaling and gene regulatory networks, systems biology can help identifying strategies to overcome resistance to vessel normalization and stratifying patients into those likely to respond to nanocarrier-delivered therapy.

The effects of vessel normalization treatments can vary among patients due to differences in the TME. Kano et al. showed that the efficiency of vessel normalization induced by VEGFR inhibitor, sorafenib, depends on the amount of pericyte coverage in tumor tissues.[142,143] Angiogenesis inhibitors failed to promote nanocarrier accumulation in a xenograft model with high pericyte coverage (BxPC3), whereas these inhibitors enhanced nanocarrier accumulation in the tumors of a xenograft model with relatively lower pericyte coverage (CT26) (Figure 6d). Thus, it is crucial to understand the relationship between the TME and angiogenic signaling to predict the effect of treatments intended to normalize blood vessels and identify appropriate patient-specific treatment strategies.

The TME is a complex physiological compartment, including endothelial cells of the blood vessels, pericytes, and immune cells. Various systems biology studies have constructed mathematical models for T cells, macrophages, and dendritic cells, which are the predominant cell types within TME.[144–146] With these mathematical models, researchers have predicted how these cells affect tumor angiogenesis. For instance, Norton et al. investigated how the amounts of macrophages and fibroblasts within the TME affect tumor vasculature.[147] Thus, this systems biological analysis suggests that it is possible to predict the effect of vessel normalization strategies on the basis of the amounts of specific cells within the TME. Furthermore, the parameters of the model can be changed to predict functional properties within a patient-specific TME. Wagner et al. utilized antibodies recognizing 73 surface markers to cluster various cells within the TME of breast cancer patients and then determined interactions between the clustered cells into a network model.[148] By developing patient-specific models, they identified patient-specific information in the tumor and TME. Using samples from nine patients with different stages of gastric cancer, Zhang et al. constructed patient-specific networks at a single-cell level using the single-cell RNA sequencing (scRNA-seq) and evaluated how these networks changed as tumorigenesis occurred (Figure 6e,f).[149] With these networks, they identified key regulatory cells that contribute to tumorigenesis (Figure 6g). Collectively, these examples illustrate how system biology can identify patient-specific characteristics of the TME, candidates for vessel normalization therapy, and additional targets to overcome resistance to normalization strategies. Consequently, systems biology research can assist nanomedicine by helping to personalize EPR strategies.

4.3. Using Systems Biology to Optimize Payload Selection and Sequential Payload Delivery

One cause of the low efficacy of nanodrugs is that the drugs may not match the properties of the specific patient's tumor or may not account for the dynamic changes that occur in the tumor and TME in response to treatment. Systems biology is a powerful approach to personalize treatment strategies not only by encapsulating drugs for simultaneous delivery, but also by encapsulating drugs for sequential therapy. Although identifying appropriate combinations of drugs is important, it is becoming more obvious that the schedule for treatment, including the sequence and timing between doses, is also critical for maximizing effectiveness and minimizing toxicity. By testing 10 000 combinations of 100 FDA-approved drugs in two cancer cell lines PANC1 (pancreatic cancer) and A375 (melanoma), Koplev et al. found that ≈23% of combinations show sequence dependency: 6.3% of combinations show synergism with sequential application and 16.5% of combinations show antagonism.[150] Preclinical studies indicate that targeted

inhibitor. Scale bars = 100 μm. Reproduced with permission.[142] Copyright 2009, Japanese Cancer Association, published by John Wiley and Sons. e–g) Using systems biology to explore the development of the TME during the progression of gastric cancer. Single-cell RNA sequencing of tumor tissues defines: e) cell types in the TME with high resolution and f) their expression profiles. g) Cell–cell networks and molecular networks for each of cell lineages give in-depth understanding of the TME during cancer progression. e–g) Reproduced with permission.[149] Copyright 2019, Elsevier.

therapy can be combined with conventional cytotoxic chemotherapeutic agents effectively, but effectiveness depends on the order of treatment. Goldman et al. determined that inhibition of hematopoietic cell kinase (HCK) followed by application of taxanes increased apoptosis of cells resistant to taxanes through a drug-induced phenotypic transition.[151] The same concept applies to targeted combination therapies. In some cases, treatments are effective but not tolerated by the patients. Appropriately sequenced combination therapies may overcome this limitation. In a preclinical study, Fang et al. found that combined inhibition of the poly(ADP-ribose) polymerase (PARP) with talazoparib and the kinase WEE1 with adavosertib reduced the survival of multiple cancer cell lines.[152] However, the combination caused weight loss when tested in mouse models of cancer, indicating that the combination was toxic. Encouragingly, sequential treatment in vitro with a PARP inhibitor and a WEE1 inhibitor was as effective as concurrent treatment in inducing apoptosis, regardless of the order of treatment. When tested in the mouse model, sequential treatment with the PARP inhibitor olaparib followed by the WEE1 inhibitor adavosertib preserved the antitumor efficacy of adavosertib monotherapy. Even more importantly, this sequential treatment strategy was as effective as the simultaneous combinatorial treatment but lacked the associated toxicities of the latter treatment. These studies indicate that sequential treatment can significantly improve efficacy and reduce toxicity, thus, is an effective therapeutic strategy for patients.

Nanomaterials engineering is well suited to achieving timed release combination therapies. Indeed, sequential release of various chemotherapeutic drugs has been actively studied using nanocarriers (Figure 3b, second row). An elegant example of the effectiveness of combining systems biology with nanomaterials engineering comes from research by Lee et al. and Morton et al., who identified that sequential inhibition of oncogenic signaling pathway followed by chemotherapy would maximize therapeutic effects in triple negative breast cancer (TNBC) and non-small-cell lung carcinoma (NSCLC), both of which are insensitive to chemotherapy (**Figure 7**a).[153,154] Lee et al. used a systems biological approach to better understand synergistic mechanism of the sequential administration and found that inhibition of the oncogenic pathway activated by EGFR induced changes in apoptotic signaling that converged on the apoptotic enzyme caspase-8 to render the cells sensitive to DNA-damaging chemotherapeutics (Figure 7b). To sequentially deliver specific drugs in vivo, Morton et al. developed liposomes that are loaded with the DNA-damaging chemotherapeutic doxorubicin in the core and the EGFR inhibitor erlotinib in the lipid layer of the liposome (Figure 7c) and tested these dual-loaded liposomes in mouse cancer models (Figure 7d–f). These liposomes first release erlotinib, which inhibits EGFR and rewires the apoptotic pathways to make the cells sensitive to the other drug, doxorubicin, which is released second (Figure 7d).[154]

Other groups have developed nanocarriers with different chemical compositions designed to release erlotinib and doxorubicin in a sequential manner. He et al. developed lipid-coated mesoporous silica nanocarriers that are loaded with these drugs and release them sequentially and showed that these nanocarriers were effective in lung cancer of a mouse model.[155] Zhou et al. developed a polymeric nanocarrier that can be modified more easily to control payload release than a liposomal nanocarrier and used this nanocarrier to sequentially release erlotinib and doxorubicin.[156]

Because nanocarrier payloads can be varied, others have developed nanocarriers that sequentially release siRNA and a chemotherapeutic agent or combinations of small molecule drugs. Deng et al. developed layer-by-layer formulated nanocarriers in which the negatively charged liposome was conjugated with poly-L-arginine (PLA) polycations, then incorporated with siRNAs, and finally coated with hyaluronic acids.[157] Using this layered formulation, a single nanocarrier can be loaded with a combination of siRNA and doxorubicin, which are then released sequentially. Such complex nanocarriers are effective in xenograft models for TNBC. Similarly, Hu et al. developed disulfide-linked glycolipid-like nanocarrier (CC-ss-SA) that was loaded with paclitaxel and coated with Bcl-2-targeted siRNAs.[158] The hydrophobic paclitaxel and the siRNA are released at different times: CC-ss-SA nanocarriers first released the siRNA inside the cell and then paclitaxel were released after about 7–11 h.

Wang et al. developed a multifunctional nanoparticle that releases verapamil, a calcium channel blocker and multidrug resistance (MDR) inhibitor, and mitoxantrone, a chemotherapeutic agent, to treat multidrug-resistance hepatocellular carcinoma (**Figure 8**a).[159] Using a biomineralization method, verapamil and mitoxantrone were loaded into the shell and core of a nanoparticle, respectively, resulting in faster release of verapamil than mitoxantrone from the nanoparticle (Figure 8b,c). The shell-core nanoparticle that sequentially releases these two drugs has synergistic efficacy both in killing multidrug-resistant cancer cells and limiting tumor growth, whereas conventional administration did not have much synergism (Figure 8d–h). Collectively, these studies show that nanomaterials engineering can be used to design multiple types of nanocarriers that mediate sequential release of drugs based on the physicochemical properties of the encapsulated payloads.

Sequential release can also be achieved using pH-sensitive formulations. Xu et al. developed inorganic nanocarriers composed of porous silica coated with poly (beta-amino ester) (PAE) and pluronic F-127.[160] These nanocarriers were loaded with doxorubicin in the silica core and paclitaxel in the F-127 shell. Paclitaxel was released by diffusion from the shell, but the release of doxorubicin was controlled by PAE, which functions as a pH-sensitive nanovalve for the particles. In this way, doxorubicin is slowly released at pH 7 (in the circulation); whereas it is rapidly released at pH 5 (in endosomes after endocytosis of the particle). Another pH-dependent nanocarrier formulation was developed by Li et al,[74] who loaded hollow-mesoporous silica nanospheres with a proteasome inhibitor (bortezomib) and DNA plasmids encoding p53. This combination cured a mouse model of NSCLC. Most bortezomib was released at pH 7.4, but the DNA plasmids were released slowly at this pH. After endocytosis, the low pH of the endosomal compartment accelerated the release of the DNA plasmids. This sequential delivery method has synergistic effects: Bortezomib inhibits the function of proteasome, which enhances the stability of p53 produced from the plasmid.

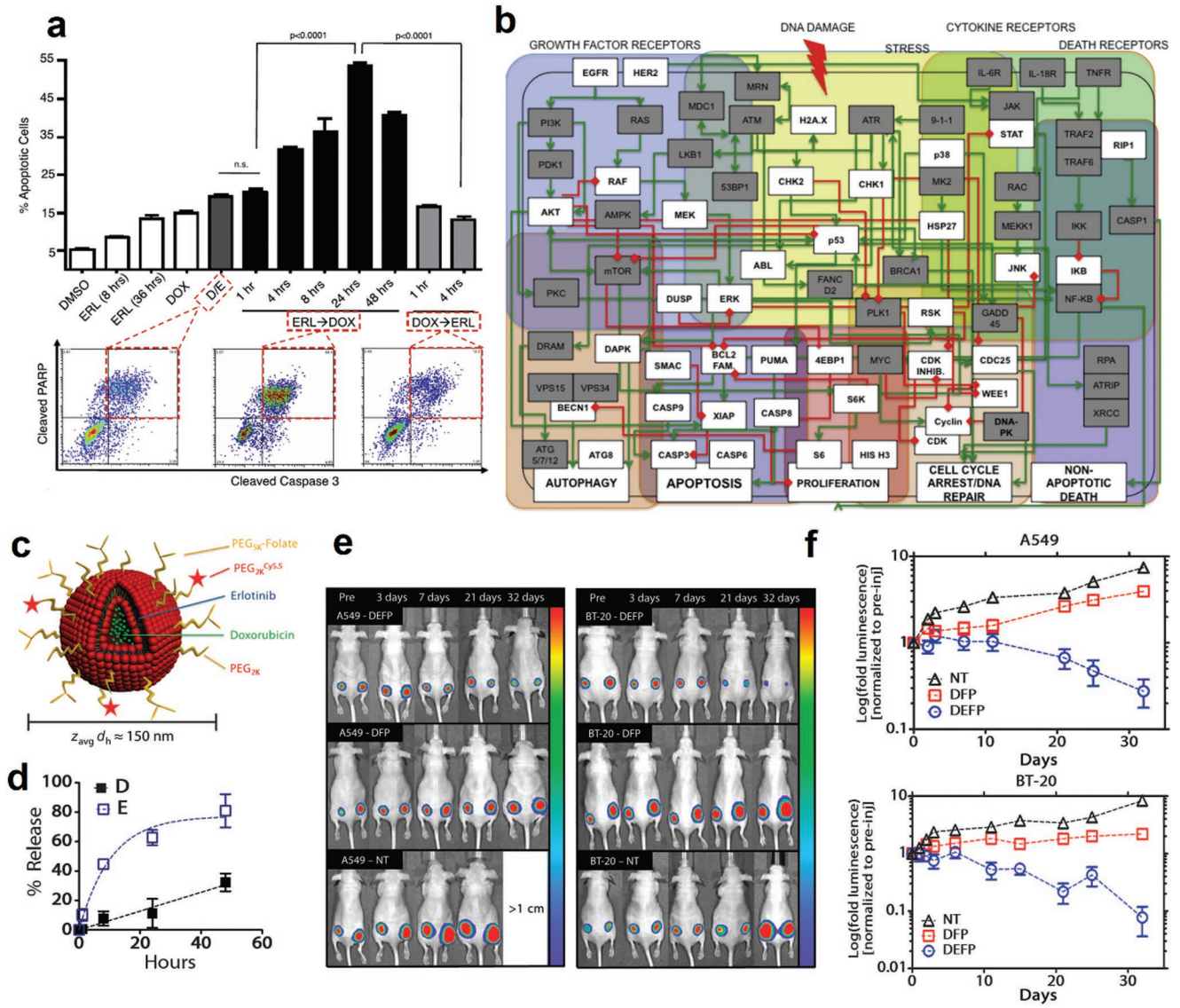

Figure 7. Integrating systems biology and nanomaterial engineering to sensitize cancer cells to DNA-damaging chemotherapy. a) Analysis of cells in culture reveals the most effective sequence of erlotinib (erl, EGFR inhibitor) and doxorubicin (dox, DNA-damaging chemotherapeutic) treatment to induce apoptosis. D/E indicates simultaneous treatment. b) Systems biological analysis of a signaling network reveals a mechanism for the identified optimal treatment sequence of erlotinib first followed by doxorubicin. Green or red arrows denote activating and repressing interactions, respectively. Proteins that were directly measured are shown in white boxes. a,b) Reproduced with permission.[153] Copyright 2012, Elsevier. c) Schematic of nanocarriers loaded with erlotinib and doxorubicin in shell and core, respectively. d) Release profiles of both drugs (D, doxorubicin; E, erlotinib) from the nanocarriers. e–f) Sequentially releasing nanoparticles loaded with erlotinib and doxorubicin are more effective at reducing tumor burden in mice with A549 or BT-20 tumors. NT, no treatment; DFP, nanoparticles with doxorubicin, folate, and PEG; DEFP, sequentially releasing nanoparticles with doxorubicin, erlotinib, folate, and PEG. c–f) Reproduced with permission.[154] Copyright 2014, American Association for the Advancement of Science.

To achieve maximum effectiveness, not only the sequence but also the timing of drug release must be carefully calibrated. For instance, if the second drug is released before the first drug reaches to its maximum efficacy, the synergistic effects of the combination cannot be achieved. Here again, nanomaterials engineering can overcome this problem. One method is incorporation of a "remote control" into the nanocarrier. For example, after administration nanocarriers can be activated by an external stimuli, such as light.[161–163] Ren et al. developed nanocarriers that sequentially release miRNA in response to pH and doxorubicin in response to near-infrared-radiation (NIR).[164] They loaded doxorubicin inside a hollow gold nanoparticle, and then attached polyamidoamine (PAMAM) around the gold nanoparticle. The miRNA was attached to PAMAM. After the nanoparticles were delivered into cells, miRNAs were released first based on pH change, and then doxorubucin was released upon NIR irradiation. Similarly, Zhang et al. developed gold nanocluster (AuNC)/Fe(OH)$_3$-poly(acrylic acid) (PAA) composite Janus nanoparticle (JNP), which has an asymmetric structure and a surface with various functions

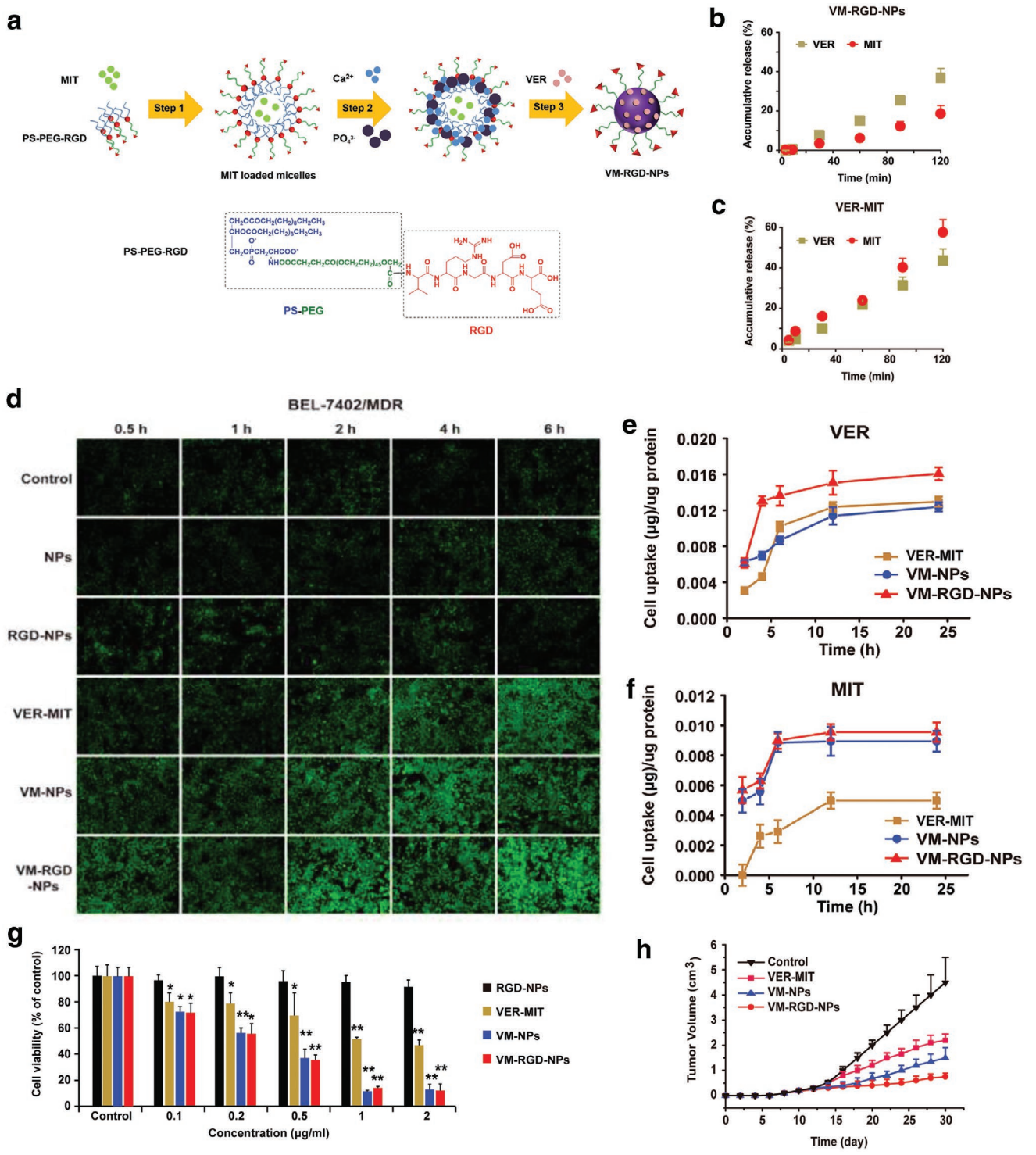

Figure 8. Sequential drug delivery by nanocarriers to overcome multidrug resistance. a) Schematic representation of synthetic steps for shell–core nanoparticles loaded with verapamil (VER) and mitoxantrone (MIT). Nanoparticles with the RGD moiety (VM-RGD-NPs) and without the RGD moiety (VM-NPs) were tested. Shell–core formulation was by the biomineralization method. b,c) Verapamil is released before mitoxantrone when delivered by the VM-RGD-NPs. The drugs accumulate with the same kinetics when delivered not encapsulated in nanoparticles (VER-MIT). d) Inhibition of the MDR efflux pump by verapamil detected by the accumulation of calcein acetoxymethyl in the tumor cell line. Compared with addition of verapamil and mitoxantrone to the culture medium (VER-MIT), inhibition occurs quickly in cells exposed to VM-NPs and VM-RGD-NPs, indicating that the nanoparticles enhanced internalization of the drugs. e,f) The VM-RGD-NPs mediate the highest delivery of verapamil (e) and either nanoparticle mediates effective delivery of mitoxantrone (f). g) Sequential release by either VM-NPs or VM-RGD-NPs was more effective than direct application of the unencapsulated drugs in inducing apoptosis of BEL7402/MDR cells. h) The VM-RGD-NPs were the most effective at limiting growth of BEL-7402/MDR tumors in nude mice. a–h) Reproduced with permission.[159] Copyright 2018, Wiley-VCH.

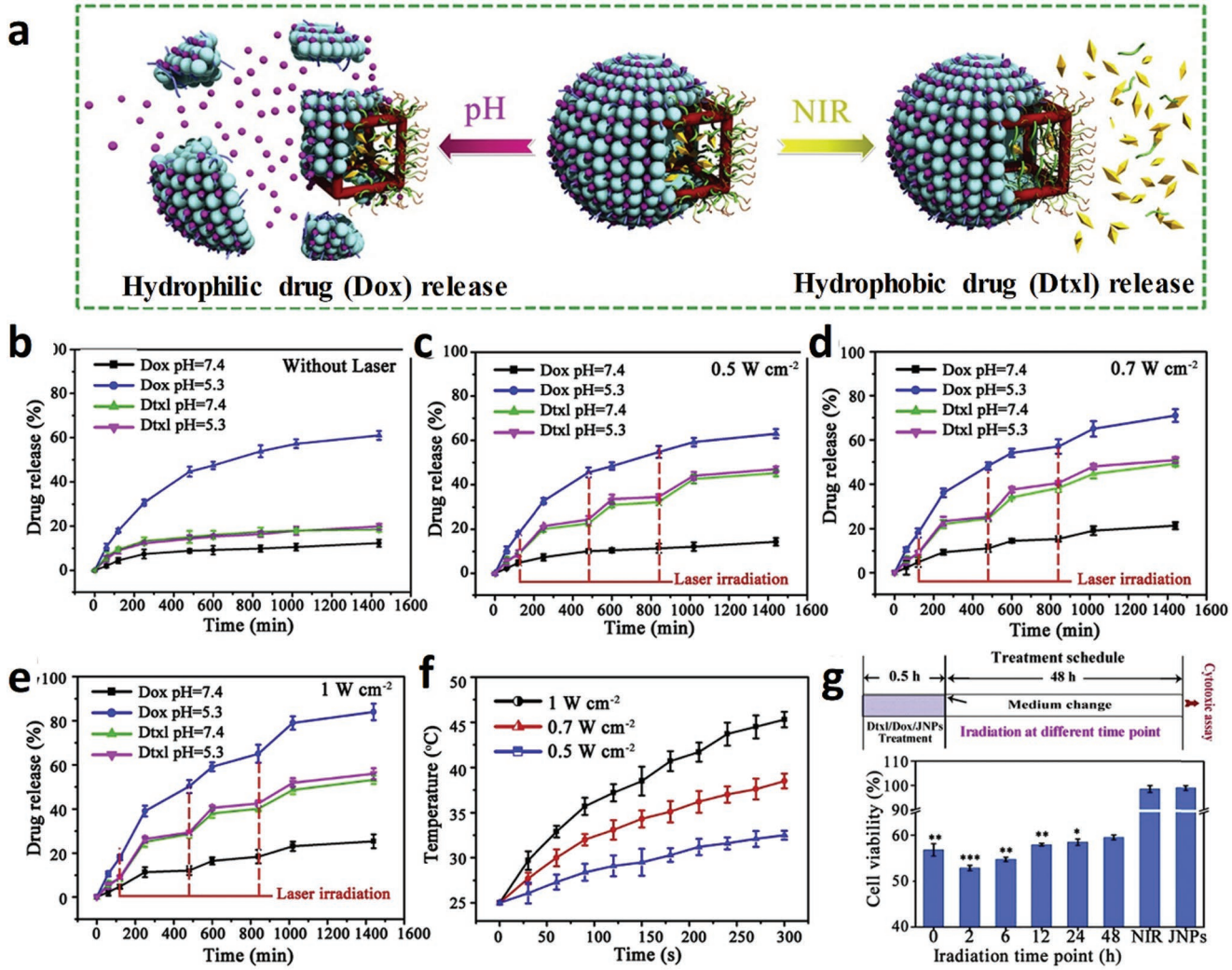

Figure 9. Precise sequential delivery by remote controlled nanocarriers. a) Schematic representation of the Janus nanoparticle (JNP), an asymmetric multifunctional nanoparticle that releases drugs in response to exposure to low pH or near-infrared radiation (NIR). JNP was loaded with doxorubicin (Dox) released by low pH and docetaxel (Dxtl) released by NIR. b–e) Release profiles of each drug from JNP under different pH conditions or intensities of irradiation. The release profile of Dtxl is precisely modified by the laser signal. f) The temperature change of the nanoparticle in response to different intensities and durations of irradiation. g) The effect of exposure of HepG-2 cells to JNP for 30 min, followed by NIR (0.5Wcm^2, 5 min) at 0, 2, 6, 12, 24, 48 h later. NIR 2 h after addition of the JNP produced the greatest reduction in cell viability. Note that NIR in the absence of the drug-loaded nanoparticle had no effect on viability, nor did JNP lacking any drug payload. a–g) Reproduced with permission.[165] Copyright 2018, Elsevier.

(**Figure 9**a).[165] These nanocarriers were loaded with hydrophilic doxorubicin and hydrophobic docetaxel in the $Fe(OH)_3$-PAA and AuNC portions, respectively. Each part of the JNP has a different property of drug release. The PAA portion is sensitive to changes in pH and releases doxorubicin in acidic conditions (Figure 9b). NIR degrades the AuNC portion due to photothermal effects, thereby releasing docetaxel in response to the radiation (Figure 9c–f). The authors adjusted the time interval of sequential release and found that the synergistic effect of sequential release was maximized when the interval was 2 h. Collectively, these studies show how nanocarriers can be engineered so that the timing of cargo release can be optimized and externally controlled.

Although sequential treatment strategies can improve drug efficacy, reduce toxicity, and overcome drug resistance, identifying optimal sequential treatments is still very difficult because of the many possible combinations to test. Although Koplev et al. generated 250000 data points representing sequential combinations of 100 FDA-approved drugs,[150] such exhaustive combinatorial testing is often unfeasible. Systems biological approaches limit the number of combinatorial options to experimentally test by identifying those most likely to produce the desired outcome. Indeed, several approaches have been developed to tackle the question of intervention sequence. Wang et al. introduced an attractor network of nonlinear model based on ordinary differential equations (ODE),[166] and Rafimanzelat et al. developed an attractor-perturbed state transition graph (APSTG) of a Boolean model.[167] Both the ODE attractor network and APSTG determine whether each attractor is reachable by a certain perturbation. Using these mathematical modeling approaches, the sequence of perturbations to reach

a desired state from an initial state can be identified. For example, sequences of inhibitors that produce cell death can be identified. Using Boolean network models, Yang et al. developed an algorithm to identify targets regulation of which drives every initial state to a steady state.[168] After converging to a certain steady state, other algorithms can be used to test the effect of perturbing additional targets to shift the steady state into the desired state. This type of sequential control can ensure homogeneous drug responses for heterogeneous initial conditions, which are present in most cancers and certainly exist between patients. Therefore, systems biological approach can identify an optimal order and combination of drugs and inform the nanomaterials engineers in the development of nanocarriers that produce the most effective sequential delivery of payloads.

5. Conclusion and Outlook

We have reviewed the recent progress and challenges in systems biology and nanomedicine in view of cancer precision medicine. Systems biology can unravel complex regulatory mechanisms underlying drug resistance of cancer cells and identify novel drug targets and treatment strategies to overcome drug resistance. However, the newly discovered drug targets are often undruggable, and conventional drug delivery systems are not suitable for implementation and testing in clinical trials. Nanomaterials engineering to develop customized nanocarriers can solve these problems by incorporating gene-targeted therapies and precise drug release (**Figure 10**a). A challenge in the successful application of nanomedicine is low efficacy due to heterogeneity of cancer, such that personalized nanomedicine is needed. Systems biological approaches can resolve issues in heterogeneity, thereby aid in developing personalized nanomedicine (Figure 10b). Hence, integration of systems biology and nanomaterial engineering can overcome the problem of drug resistance and heterogeneity in cancer patients, and realize the power of precision medicine for the treatment of cancer.

One challenge in personalized nanomedicine that we have not reviewed is toxicity of nanoparticles. Nanoparticles can be cytotoxic by inducing the production of reactive oxygen species (ROS) in cells and immunogenic by inducing pro-inflammatory signals.[169] Mechanisms of toxicity and immunogenicity of nanoparticles vary according to their material, size, zeta-potential, surface chemistry, and morphology.[170] With this complexity, it is not surprising that the detailed mechanisms for cytotoxicity and immunogenicity of diverse nanoparticles have not been determined. Systems biological approaches can predict side effects and drug toxicity for conventionally delivered medicines.[171,172] Not surprisingly, systems biological studies have provided insights into the mechanisms of toxicity and immunogenicity of some nanoparticles.[173–176] Therefore, we anticipate that systems biology will help overcoming the challenges related to nanoparticle toxicity.

Nanomaterial engineering has been applied to overcome some of the limitations associated with cancer immunotherapy. In particular, nanomaterial engineering has been actively applied to alleviate the problem of limited efficacy and the induction of adverse effects due to nonspecific activation of immune responses.[10,111,177,178] Considering that the immune responses result from complex interactions between cancer cells and TME, systems biology can also help in identifying right target cells and molecules for combinatorial therapy to further improve immunotherapy delivered with nanomaterials.[179]

Cancer precision medicine presents its own set of specific challenges mostly related to heterogeneity across patients and even within the tumor cell population in a single patient. Intratumor heterogeneity causes different responses to the same anticancer therapy within a single cancer patient. Because tumor cell heterogeneity is mostly caused by genetic mutational differences, each subtype of cancer cells needs to be represented by a different network model to most effectively predict responsiveness to any particular therapy. Systems biological analysis can identify optimal targets within each network model, and nanocarrier technology can achieve delivery of the therapy specifically designed for each subtype of cancer cells. Thus, not only adaptive drug resistance but also intratumor heterogeneity can be overcome by applying the joint forces of systems biology and nanomaterial engineering.

Clinical trial design is another place where systems biology and nanomaterial engineering can be effectively applied. Clinical trials are categorized as umbrella trials or basket trials. In basket trials, cancer patients with a common biomarker are prescribed the same treatment independent of their cancer types, because drug efficacy is assumed to be determined by the shared biomarkers. In contrast, in umbrella trials, patients are treated according to their cancer types, because drug efficacy is assumed to be tissue specific. Different systems biological approaches and nanocarriers can be designed for each type of clinical trial. For basket trials, the systems biological approach would use a pan-cancer model to identify optimal drug targets that are independent of cancer types. With this model, different genetic variations and underlying pathology can be tested. For umbrella trials, a tissue-specific cancer model is required. Likewise, basket trials require a drug delivery system that can deliver drugs to cancer cells arising in any part of the body; whereas umbrella trials require a cancer-type specific drug delivery system. Furthermore, it is possible to design a novel clinical trial by combining cancer type-independent drug targets from a pan-cancer model with cancer type-specific nanocarriers for each patient.

To integrate systems biology and nanomaterial engineering for cancer precision medicine more effectively, systems biologists should consider at the beginning of the study the actual number of drugs that can be loaded in a single nanocarrier. Because the prediction from the result of perturbing targets is often qualitative, systems biology studies should provide quantitative criteria for nanoengineers to examine whether a developed nanodrug works comparably to that predicted from network model simulation. Close interaction between systems biology and nanomaterial engineering will open a new way to overcome the current limitations of molecularly targeted therapy and provide an innovative strategy for precision medicine.

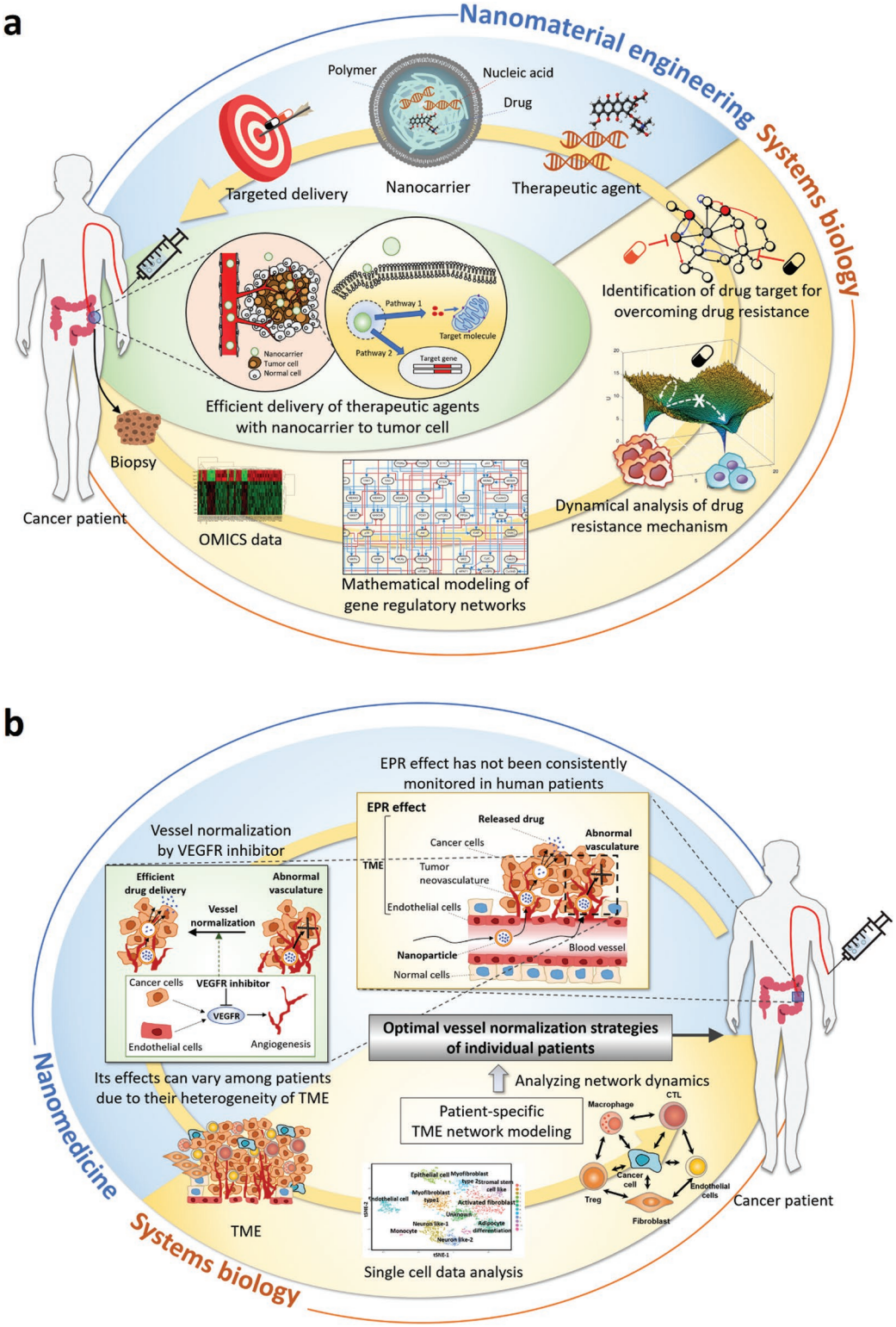

Figure 10. Integration of systems biology and nanomaterial engineering to overcome the challenges of precision medicine. a) Systems biology can identify novel drug targets for overcoming drug resistance using omics data of cancer patients and dynamical analysis of cellular regulatory networks. These targets can be difficult to be inhibited through conventional mechanisms or optimal treatment requires sophisticated delivery and scheduling paradigms. Nanomaterial engineering can develop nanocarriers to specifically deliver therapeutic agents to the cancer cells for the novel targets, including those that cannot be easily targeted through conventional methods. Thus, the integration of the two fields achieves cancer precision medicine by overcoming the challenges associated with the targets identified by systems biology. b) Presently, nanomedicine is limited by low efficacy due to heterogeneity in patient EPR. Systems biological approaches can resolve this issue by identifying optimal targets for vessel normalization and thus contribute to improving the efficacy and delivery of personalized nanomedicine.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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