Application of synthetic biology in bladder cancer

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Abstract

Bladder cancer (BC) is the most common malignant tumor of the genitourinary system. The age of individuals diagnosed with BC tends to decrease in recent years. A variety of standard therapeutic options are available for the clinical management of BC, but limitations exist. It is difficult to surgically eliminate small lesions, while radiation and chemotherapy damage normal tissues, leading to severe side effects. Therefore, new approaches are required to improve the efficacy and specificity of BC treatment. Synthetic biology is a field emerging in the last decade that refers to biological elements, devices, and materials that are artificially synthesized according to users' needs. In this review, we discuss how to utilize genetic elements to regulate BC-related gene expression periodically and quantitatively to inhibit the initiation and progression of BC. In addition, the design and construction of gene circuits to distinguish cancer cells from normal cells to kill the former but spare the latter are elaborated. Then, we introduce the development of genetically modified T cells for targeted attacks on BC. Finally, synthetic nanomaterials specializing in detecting and killing BC cells are detailed. This review aims to describe the innovative details of the clinical diagnosis and treatment of BC from the perspective of synthetic biology.

Keywords: Bladder cancer; Synthetic biology; Gene circuits; T cell immunotherapy; Nanomaterials

Introduction

Bladder cancer (BC) is the 10th most common malignancy, with an estimated 573,000 new cases diagnosed and 213,000 deaths noted worldwide in 2020.[1] Tobacco smoking is generally considered the strongest risk factor for the development of BC. In addition, occupational exposure to aromatic amines and some industrial chemicals, as well as chronic inflammatory conditions, have been implicated in BC risk.[1,2] Non-muscle-invasive BC (NMIBC) accounts for approximately 80% of BC cases. The front-line treatment for NMIBC is transurethral resection of bladder tumor (TURBT), followed by intravesical chemotherapy or immunotherapy such as pirarubicin or Bacillus Calmette–Guérin (BCG). Although the survival rate is favorable, NMIBC frequently recurs and requires patients to undergo long-term surveillance with cystoscopy.[2,3] Patients with muscle-invasive BC (MIBC) comprise approximately 20% of the BC population. Radical cystectomy (RC) is the mainstay of surgical treatment. Cisplatin-based chemotherapy and radiation are occasionally performed to lower the risk of metastasis and disease-specific mortality.[3] To date, BC research through traditional molecular biology and next-generation sequencing has already revealed the genomic landscape and the molecular underpinnings of BC.[3] Cancer cells have unique features that allow us to distinguish them from normal cells, for example, the prevalence of activating mutations in the telomerase reverse transcriptase (*TERT*) promoter, inactivating mutations in tumor protein p53 (*TP53*), and increased intracellular reactive oxygen species (ROS) and glutathione (GSH).[3-5] Available BC-related markers and actionable therapeutic targets are the basis for synthetic biology applications in cancer research.

Synthetic biology is an emerging field that aims to artificially design and construct novel biological elements, devices, and systems based on a researcher's needs, such as exploiting novel diagnostics and therapeutics.[6,7] Over the past decade, the field of synthetic biology has been developing rapidly due to the expansion of powerful genetic engineering tools, the reduced costs of nucleic acid

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synthesis, and high-throughput sequencing. Currently, synthetic biologists can program mammalian cells, bacteria, and other materials with artificial gene circuits[6-10] and endow them with the ability to perceive the characteristics of the tumors, trigger internal sophisticated signaling pathways and integrate this information to produce therapeutic outputs that can be regulated exogenously. Using this methodology, researchers can freely set and precisely control treatment time, duration and location. In addition, synthetic biomaterials, such as nanomaterials, are also widely used in the diagnosis and treatment of BC given their super biocompatibility, reconfigurability, adhesion, and drug delivery properties.[11,12]

This review discusses the research progress and clinical prospects of synthetic biology in BC. We begin by elaborating how synthetic gene elements and gene circuits are designed and assembled to regulate the expression of BC-related genes and differentiate malignant cells from normal cells. Next, we review advances in modified immune cells and ongoing clinical trials in BC. We conclude by demonstrating various means to prepare nanomaterials for diagnosing or treating BC. Taken together, synthetic biology provides precise, condition-specific control over tumor-specific recognition and cellular behavior, revolutionizing the detection, treatment, and research approaches of BC.

Nucleic Acid-Based Tumor Recognition and Gene Therapies in BC

Nucleic acid-based synthetic biology programs living cells with synthetic genetic elements or artificial gene circuits that allow cells to sense and respond to specific signals and then autonomously perform user-defined biological functions.[13,14] Currently, CRISPR/Cas systems are widely used in synthetic biology for gene expression regulation and gene circuit construction due to their high specificity, robustness, and versatility. Synthetic biological circuits typically consist of three components: inputs, processors, and outputs. Specifically, the inputs can be exogenous such as user-provided small molecules, or endogenous such as tumor-specific transcripts or proteins. The processors are required to be capable of integrating multiple input signals and triggering desired gene regulation, whereas the outputs force cell behavior through a different mechanism, such as the inhibition of oncogenes or activation of tumor suppressor genes.[7,13]

Gene Editing Tools Derived from CRISPR-Cas Systems

CRISPR was discovered in the adaptive immune system of bacteria, archaea, and huge bacteriophages.[15,16] The first editing tool to be identified is CRISPR–Cas9 derived from *Streptococcus pyogenes* (*SpCas9*). The mechanism of CRISPR/Cas9 is that after scanning protospacer adjacent motif (PAM) sequences, single guide RNAs (sgRNAs) recognize the complementary oligonucleotide sequence on the target gene, and Cas proteins cleave a specific locus, resulting in DNA double-strand breaks. The subsequent non-homologous end-joining repair of the process of the double-strand break could lead to mutations or deletions in the genome to silence the target gene.[17] However, later studies found that the action mode of Cas9 could be switched between DNA cleavage or just binding by fine-tuning the length of the sgRNA.[18] In recent years, other types of Cas9 orthologs, such as Cas12, Cas13, Cas14, Cas X, and CasΦ, have also been identified, some of which have reduced molecular sizes and improved targeting specificities.[16,17,19] Collectively, the ongoing evolution of CRISPR/Cas systems provides a unique and versatile toolbox for genome and epigenome manipulation.

CRISPR-Cas systems have been redesigned to improve the target specificity, endonuclease activity, or delivery efficiency of this system. The nuclease cleavage activity of CRISPR/Cas proteins can be inactivated by site-specific mutation, termed dead Cas (dCas). This mutation allows the proteins to be repurposed as a programable DNA-binding module and conjugated with diverse effector domains, including transcriptional activators, repressors, and epigenetic modifiers.[20,21] The modified CRISPR/dCas system is diffusely employed for the regulation of BC-related genes; for example, Cao *et al*[22] fused dCasX to the transcriptional repressor domain Krüppel-associated box (KRAB) to silence oncogene *c-Myc* and to VPR to upregulate the tumor suppressor gene *TP53* to inhibit the development of BC. In addition, a study integrated the RNA modification N6-methyladenosine (m6A) demethylase fat mass and obesity-associated protein (*FTO*) into the C-terminus of dCas13b to construct a targeted *c-Myc* demethylation system, namely, "MYCdm6A".[23] MYCdm6A has the ability to effectively inhibit *c-Myc* expression by demethylation at the mRNA level. The researchers demonstrated that this biodevice partially suppressed the malignant phenotypes of BC cells.

Specific Identification for BC

Several studies focused on identifying BC with cancer-associated transcription factors (TFs) or promoters. One example of a genetic sensor for BC is based on *TP53*.[24] They identified p53-bound enhancer regions (P53BERs), which were specifically recognized and bound by p53, and placed P53BER upstream of the SV40 promoter. P53BER-SV40 components were used to drive sgRNA and Cas9 expression. They transferred the *TP53* genetic sensor and vectors expressing diphtheria toxin into cells. In healthy cells that stably express p53, p53 bind to a specific site within P53BER, which then drives CRISPR/Cas9 expression to cleave diphtheria toxin and abolish its cellular toxicity. However, in tumor cells, such as the BC cell line 5637, p53 is not expressed, and diphtheria toxin is highly expressed, resulting in cell death.

Another approach to differentiate BC cells from other cells involves gene circuits with a dual-promoter integrator (DPI) [Figure 1A]. Researchers employed the bladder-specific human uroplakin II (*hUP II*) gene promoter to drive the expression of Cas9, and the cancer-specific human TERT (*hTERT*) promoter to drive the transcription of sgRNA to construct a modular AND gate circuit.[25] Theoretically, only BC cells, not other tumor cells and urothelial cells, simultaneously have large amounts of *hUP II* and *hTERT*. After the above two

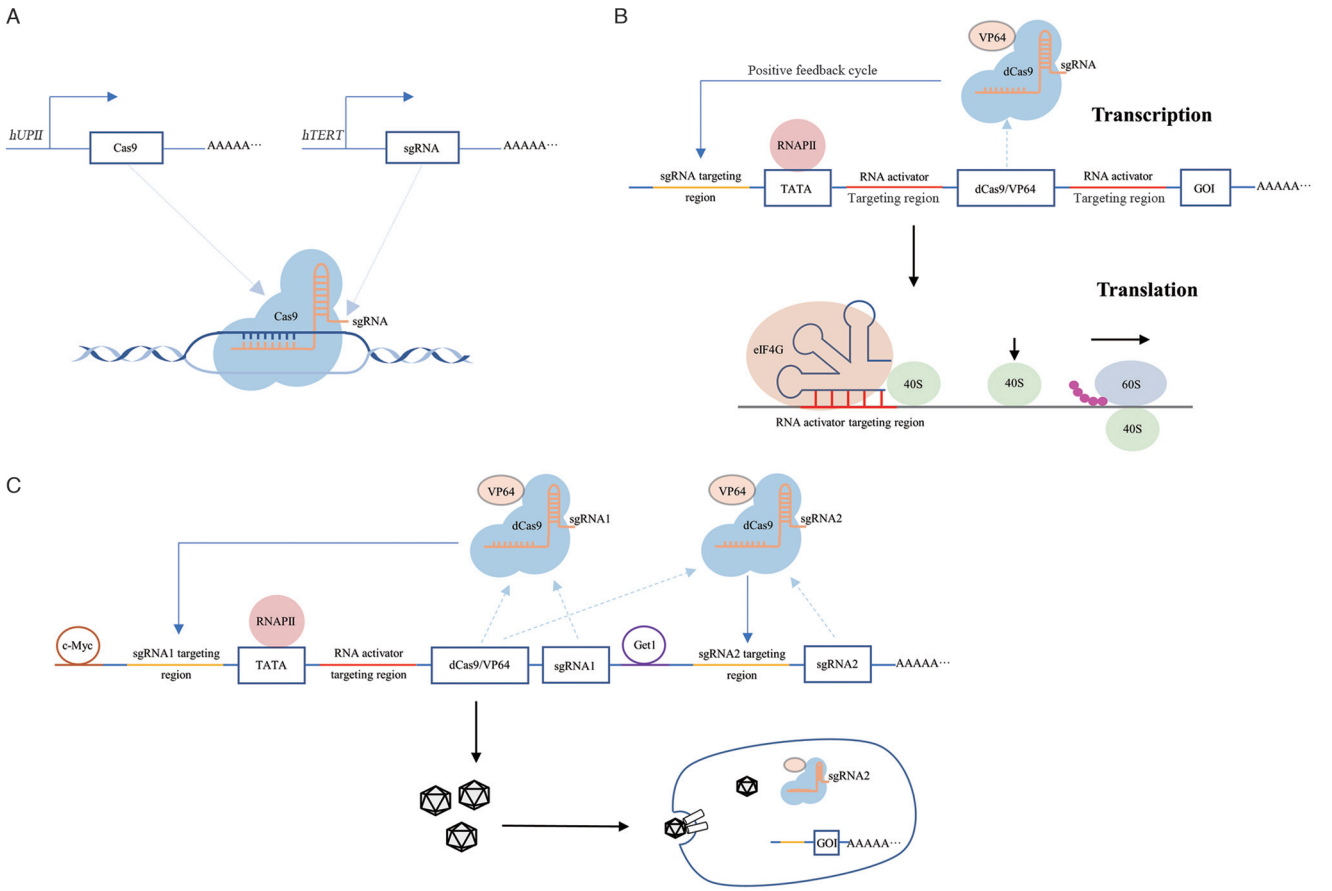

Figure 1: Synthetic gene circuits for specific recognition and gene regulation in BC. (A) The *hUP II* AND *hTERT* logical gate circuit. Cas9 and sgRNA transcription is driven by *hUP II* and *hTERT* promoters, respectively. Only when *hUPII* and *hTERT* exist simultaneously can the CRISPR/Cas9 sgRNA system function effectively. (B) Working principle diagram of CRISPReader. In the whole gene circuit, a traditional promoter is deleted, leaving only the TATA box. Then, a sgRNA-bound region is placed upstream of the TATA box, to which dCas9/VP64-sgRNA can be directed to enhance transcription with positive feedback. Subsequently, eIF4G binds to the aptamers upstream of the GOI and then recruits ribosomal subunits (40S, 60S) to initiate translation events. (C) *c-Myc* AND *Get1* logical gate circuit based on CRISPReader. CRISPReader technology is employed to construct a *c-Myc* AND *Get1* logical gate circuit to reduce the size of the gene circuit so that it can be packaged into the AAV vector. CRISPR/Cas9-sgRNA2 is responsible for outputting the effector signal of the gene circuit. The presence of the positive feedback system controlled by *c-Myc* and *Get1* can better differentiate BC cells from other cells. AAV: Adeno-associated virus; BC: Bladder cancer; dCas: Dead Cas; eIF4G: Eukaryotic initiation factor 4G; GOI: Gene of interest; *hTERT*: Human *TERT*; *hUP II*: Human uroplakin II; *RNAP II*: RNA polymerase II; sgRNA: Single guide RNA.

systems are transferred into cells, the CRISPR/Cas system functions to output signals only when both *hTERT* and *hUP II* promoters are activated simultaneously. These signals can distinguish BC cells from other cells. In addition, the outputs can be set arbitrarily, and reporter genes can be used to verify whether the system works as well as BC-related genes to alter the malignant abilities of BC cells. In brief, DPI allows the specific recognition and efficacious inhibition of BC cells.

Once the gene circuits are successfully constructed, it is important to consider how to deliver them into the body. In the era of gene therapy, adeno-associated virus (AAV) is the most promising vector among therapeutic gene delivery platforms given its safety, excellent efficacy, and compatibility with mass production. Of note, AAV is currently the only US Food and Drug Administration (FDA)-approved vehicle for *in vivo* gene transfer, and numerous clinical trials have been completed or are ongoing.[26] In this regard, AAV-loadable CRISPR-based gene circuits have great potential in the detection and treatment of BC *in vivo*. However, due to the large size of Cas proteins and the limited payload size of AAV (<4.7 kb), CRISPR/Cas9 systems are difficult to package into AAV particles for primary cells and *in vivo* delivery.[27] In previous studies, SpCas9 (4.2 kb) was divided into two parts before being packaged into AAV separately.[28,29] However, these dual AAV systems reduce the delivery efficiency and biological activity of Cas proteins. Therefore, simplifying the gene circuits becomes a crucial issue. In this case, CRISPReader, a technology for controlling transcription and translation initiation efficiently without a canonical promoter,[30] was developed [Figure 1B]. The CRISPReader system retained only the minimal TATA box of the promoter, which can be bound by RNA polymerase II (RNAP II). dCas9/VP64-sgRNA, which targets upstream of the minimal TATA box, was employed to amplify transcriptional signals of the whole gene circuits. Then, RNA activators containing the aptamers for eukaryotic initiation factor 4G (eIF4G) are bound to their corresponding targeting regions upstream of the target genes to facilitate translation. Here, eIF4G was used to recruit ribosomal subunits and prompt the formation of initiation factor complexes. After removing redundant traditional promoter regulatory sequences and translation initiation elements, gene circuits constructed

by CRISPReader can be delivered to cells using the all-in-one AAV vector.

Subsequently, researchers applied CRISPReader to optimize the abovementioned DPI gene circuits, in which the *UPII* and *TERT* promoters were replaced by their respective TF binding elements. Previous studies have confirmed that the transcriptional activities of *hTERT* and *hUPII* promoters are mainly regulated by the TFs *c-Myc* and *Get1*, respectively [Figure 1C]. Based on the design concept of CRISPReader technology, the binding of TFs (*c-Myc* and *Get1*) and their corresponding elements promotes the transcription of the entire gene circuit in a positive feedback manner, amplifying the activation effects of *c-Myc* and *Get1*. Thus, a small amount of CRISPR/Cas9-sgRNA is sufficient to drive and enhance downstream gene transcription by further binding upstream of the TATA box. Therefore, compared with normal cells and other tumor cells, the gene circuit can be strongly activated in BC cells under the control of *c-Myc* and *Get1*. CRISPR/Cas9-sgRNA2 is responsible for executing the effector function of the gene circuit to regulate BC-related genes in cells. The cancer diagnostic and therapeutic efficacy of gene circuits based on CRISPReader were much better than that of the traditional gene circuits. Moreover, the CRISPReader system is 3.2 kbp smaller, allowing packaging into AAV vectors.[31]

Gene Regulation in BC

Synthetic gene switches

Genetic elements that respond to exogenous (such as tetracycline and theophylline) or endogenous (such as *TERT*) signals have been established. Researchers use these elements to artificially control the expression of some star oncogenes, thereby suppressing the malignant phenotype of BC.[32-34] Furthermore, a light-inducible gene expression device has been devised. This type of system fuses the transcriptional activation domain (AD, e.g., VP64, P65, etc.) to cryptochrome 2 (CRY2) and dCas9 to CIB1 (binding partner of CRY2). Without blue light, the genomic anchor binds to the targeted promoter with the guidance of the sgRNA, whereas CRY2-AD is freely diffused in the nucleus. In the presence of blue light, CRY2 and CIBI are heterodimerized, recruiting transcriptional ADs to the CIBI-dCas9 to activate tumor suppressor genes.[35] Optogenetic gene expression systems enable spatiotemporal regulation of gene transcription to influence tumor cell behavior.

Construction of gene circuits that reverse cancer-promoting signaling in BC

To achieve multiple sophisticated signal integration and intracellular processing, one study modified CRISPR/Cas sgRNA with signal-responsive aptamers named CRISPR signal conductors[36] [Figure 2A]. In the absence of the ligand, sgRNA is blocked with the antisense stem of the aptamer and cannot access its target DNA. Once the ligand is present, it can interact with the aptamer and induce a conformational change that allows the sgRNA to bind to the target gene. CRISPR signal conductors link input signals to the regulation of gene expression events, all of which can be user-defined. The inputs not only include small-molecule drugs (such as tetracycline or doxycycline) but also oncogenic signals within BC (such as nucleophosmin [NPM] or Ets-1), whereas output effectors include the activation of tumor suppressors and the inhibition of oncogenes. Based on the functions of different combinations of sgRNAs, various Boolean logic gates can be constructed by the signal conductors to redirect oncogenic signaling to an antioncogenic pathway, thereby specifically killing BC cells. Similarly, CRISPR/Cpf1-based transcriptional regulatory devices were also developed.[37]

Due to the limited number of aptamers available, researchers exploited CRISPR signal conductor version 2.0 based on the functional long non-coding RNAs (lncRNAs) motifs. CRISPR signal conductor version 2.0 works by integrating multiple lncRNA functional motifs into different sites of crRNA (CRISPR RNA) in the CRISPR-dCasΦ system[38] [Figure 2B]. For example, aptamers for intracellular protein signals can be integrated into the 3′-end DNA recognition region of crRNA to determine whether the crRNA functions. In addition, lncRNA motifs that bind to transcriptional activators or transcriptional repressors were inserted at the 5′ end of crRNA. The results showed that this system had good performance in both endogenous and reporter gene regulation, and was not inferior to that of dCas9-VPR and dCas9-KRAB. The middle stem–loop region of crRNA can also be fused with some lncRNA motifs to regulate crRNA activity by combining with endogenous proteins [Figure 2B]. In conclusion, CRISPR signal conductor version 2.0 can handle logic gates with as many as six inputs [Figure 2C], avoiding background leakage and increasing the specificity of tumor recognition. In addition, they used the CRISPReader technology described above to achieve specific high expression of dCasΦ in BC cells *in vivo*. It is worth noting that this system had no significant effect on the immune system and survival time of mice, and this strategy inhibited the growth of both subcutaneously transplanted BC tumors and lung metastasis.

An aptamer-dependent translational regulatory platform consisting of a series of RNA devices was designed as well.[39] Given that inserting an RNA aptamer into the 5′ untranslated region (5′-UTR) of mRNA can block ribosomal scanning to restrain the translation of mRNA when its ligand exists, Liu and his colleagues developed "signal-connector." A negative signal connector composed of a 20-nt antisense RNA complementary to the target gene and aptamers alone can disturb translational events performed by the ligand-aptamer complex, whereas the positive signal connector includes eIF4G aptamers. Two types of aptamers are present in positive signal connectors. One type changes conformation upon ligand binding to help the signal connector to localize to target genes, and the other is used to recruit eIF4G to facilitate the translation of target mRNAs. These connectors sense various tumor signals and output tumor suppressor signals as described previously to realize the transition

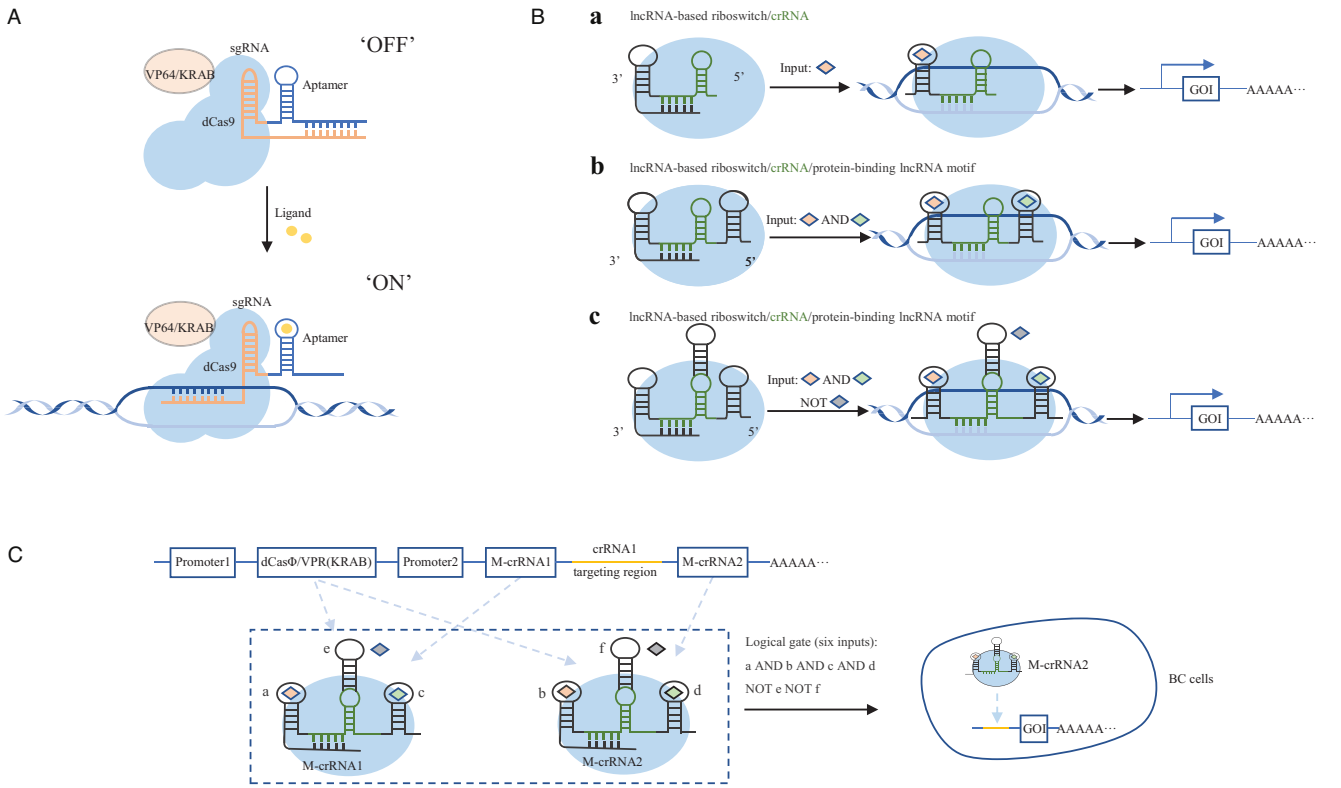

Figure 2: Gene circuits that sense multiple tumor-derived signals and present tumor-suppressive outcomes. (A) CRISPR signal conductors (version 1.0). When the ligand exists, the conformation of the aptamer is changed, and sgRNA can function to guide the dCas9 protein to the position of the target gene. Otherwise, sgRNA will not work when blocked by the aptamer. (B) CRISPR signal conductor (version 2.0). a. A lncRNA-based riboswitch is placed on the 3′ end of crRNA to ensure that the crRNA will only function in the presence of an input signal; b. In addition to the lncRNA-based riboswitch, a protein-binding lncRNA motif that recruits transcriptional activators or repressors (input) is integrated into the 5′ end of crRNA. When the input signals are present, gene expression will be greatly affected; c. A lncRNA motif is additionally inserted into the middle stem–loop region of crRNA. Here, the presence of a ligand prevents the crRNA from functioning; (C) CRISPR signal conductor version 2.0 allows the handling of the logic gates with six inputs if crRNA1 is designed to regulate crRNA2 transcription. dCas: Dead Cas; GOI: Gene of interest; KRAB: Krüppel-associated box; lncRNA: Long non-coding RNA; sgRNA: Single guide RNA.

from promoting tumor states to inducing anticancer pathways in cells.

Most of the synthetic gene circuits in BC are designed through the CRISPR/Cas system. To date, clinical trials based on CRISPR/Cas9 gene editing have been carried out in transthyretin amyloidosis,[40] sickle cell disease and β-thalassemia,[41] HIV and acute lymphocytic leukemia.[42] The primary results are encouraging. Synthetic biology-derived gene switches and gene editing technologies that sense multiple tumor signals and precisely control therapeutic interventions hold great promise in BC clinical management. However, the application of synthetic biology in BC is still in the laboratory stage. There are no clinical trials to evaluate the safety and efficacy of these gene circuits. More efforts will be required before synthetic biology is allowed into the clinic.

Cell-Based BC Therapies

Synthetic gene circuits can also be delivered to immune cells, directing them to attack tumors. At present, cancer immunotherapy is one of the most promising anticancer therapies. Numerous studies have shown that T cells with genetically modified T-cell receptors (TCRs) or chimeric antigen receptors (CARs) exhibit good antitumor performance.[43-45] In general, T cells are collected from patients and activated *in vitro* with antibodies against CD3 and CD28. Then, lentiviruses or retroviruses are preferentially used to integrate the expression vectors of TCR/CAR into the genome of T cells, allowing the construction of durable TCR-T/CAR-T cell populations. After several weeks of *in vitro* culture, these T cells are reinfused into the patient, where they can specifically recognize and lyse cells possessing the tumor antigen.[46]

Design and Optimization of TCRs and CARs

TCR is a heterodimer composed of α and β peptide chains, and each peptide chain contains a variable region domain, a constant region domain, and a transmembrane region. The variable region domain interacts with the antigen peptide presented on the major histocompatibility complex (MHC) and determines the antigenic specificity of TCRs. TCR forms complexes with multiple CD3 signaling subunits (such as CD3γ, CD3ε, and CD3δ) for signal transduction to activate T cells.[45] Moreover, TCRs responsive to specific tumor antigens are typically obtained from tumor-infiltrating lymphocytes. CARs are designed to recognize specific tumor surface antigens through a single chain variable fragment (scFv) derived from the monoclonal antibody. The first-generation CARs consist of a scFv, an intermediate hinge region, a transmembrane domain, and an intracellular signaling

domain derived from the TCR. Second- and third-generation CARs include additional one and two costimulatory domains, respectively, in addition to the above components. Generation I, II, and III CARs differ based on the number of costimulatory domains they contain, whereas generation IV CARs differ in their ability to recruit other immune cells through further genetic engineering.[44] The main difference between TCR-T and CAR-T cells is that the antigen targeted by TCR-T cells can be located in the cytoplasm or cell membrane, whereas the function of TCR-T cells depends on MHC.[45]

CAR-T cell therapy has demonstrated high rates of total remission when applied in patients with hematological cancers, and several CAR-T cell products targeting CD19 have been approved by the FDA for clinical use.[44] However, CAR-T cell therapy for solid cancers has encountered challenges. First, solid tumors lack ideal targetable antigens. Given that most tumor-associated antigens have a low or moderate expression in normal tissues, the on-target and off-tumor effects are inevitable in some cases of CAR-T-cell applications. Second, it is relatively difficult for CAR-T cells to access tumor cells due to the rich matrix of tumor tissues. More importantly, immunosuppression of the tumor microenvironment which contains immunosuppressive cells and cytokines, such as tumor-associated macrophages (TAMs) and immunosuppressive regulatory T cells (Tregs), TGF-β and IL-10, also hinders the killing function of CAR T cells.[47] Recently, two interesting studies have revealed the difference in the killing mechanisms of CAR-T against solid tumors and liquid tumors. Charly and colleagues discovered that in solid tumors, prolonged antigen stimulation promoted the transformation of CD8+ T cells to NK-like T cells, leading to CAR-T cell exhaustion. The upregulated TFs ID3 and SOX4 are responsible for the expression of exhaustion-related genes and NK receptors during CAR dysfunction.[48] Another study found that abnormalities in the interferon-γ receptor (IFNγR) signaling pathway in tumor cells significantly impaired the killing ability of CAR-T cells, as IFNγR1 loss hindered the formation of robust immune synapses between tumor cells and CAR-T cells. However, this phenomenon was not observed in leukemia or lymphoma.[49]

CAR and TCR Clinical Trials for BC

Several CAR-T clinical trials targeting bladder tumors, or solid tumors including BC or urothelial cancer, are currently in progress to test the efficacy and safety of T cell therapy alone or in combination with other therapies [Table 1]. A phase I/II clinical trial in China evaluated a fourth-generation CAR targeting prostate-specific membrane antigen (*PSMA*) and Fos-related antigen (*FRa*) in advanced or metastatic BC (NCT03185468). However, no BC trial results have been posted to date. Another phase I study combines oncolytic adenovirus therapy with *HER2*-specific CAR-T cells to treat advanced *HER2*-positive solid tumors, including BC. Researchers injected an oncolytic adenovirus (CAdVEC) intratumorally to create a proinflammatory tumor microenvironment that aims to promote the recruitment and expansion of *HER2* CAR-T cells. The study is now in the recruiting stage (NCT03740256). There is also a CAR-T trial targeting the tyrosine-protein kinase transmembrane receptor *ROR2*, and the safety, tolerability, and antitumor activity of the CAR-T cells are currently being evaluated. Subjects received the conditioning chemotherapy regimen of cyclophosphamide and fludarabine for lymphodepletion. Beyond the studies presented above, a dose escalation phase I study to assess the safety and clinical activity of multiple cancer indications, including BC, has also been registered. However, the results of all CAR-T clinical trials related to BC and two TCR-T trials targeting *NY-ESO-1* have not been reported.

CAR-T immunotherapy has shown great success in hematological tumors. There are also considerable studies on some solid tumors, such as glioma and breast cancer. Various optimized CAR-T programs are proposed. For example, Choe *et al*[50] utilized synNotch receptors to overcome tumor heterogeneity, and improved antitumor efficacy and T cell durability in treating glioblastoma. Moreover, tandem scFvs were utilized to build OR logic gates to avoid tumor escape in B cell acute lymphoblastic leukemia.[51,52] In addition, one study combined CAR-T cells with knockdown of immune-depleting molecules, such as *PD1*, to increase the killing ability and persistence of T cells in multiple myeloma.[53] However, research on modified T cell therapy in BC is still in its infancy, which is reflected not only by the limited available targets with unknown efficacy, but also by the lack of optimal combination therapy regimens. More attention should be devoted to the development of BC-specific targets for CAR-T or TCR-T cell therapy and the treatment strategy to improve specificity, overcome tumor escape, and optimize the therapeutic effect.

Table 1: BC-associated clinical trials of modified T cells.

Identifier	Phase	Engineering T cells	Tumor antigen	Combined treatment	Status
NCT03185468	I/II	CAR	PSMA and FRa		Unknown
NCT03960060	I	CAR	ROR2	Cyclophosphamide and fludarabine	Active, not recruiting
NCT03740256	I	CAR	HER2	CAdVEC	Recruiting
NCT03018405	I/II	CAR	NKG2D		Recruiting
NCT02457650	I	TCR	NY-ESO-1	Cyclophosphamide and fludarabine	Unknown
NCT02869217	I	TCR	NY-ESO-1	Cyclophosphamide and fludarabine	Recruiting

BC: Bladder cancer; CAR: Chimeric antigen receptor; FRa: Fos-related antigen; PSMA: Prostate-specific membrane antigen; TCR: T-cell receptor.

Nanomaterials in BC Diagnosis and Treatment

Synthetic biomaterials, such as nanomaterials, hold tremendous potential in the clinical application of BC because they possess good biocompatibility and bioadhesive and drug-loading properties. Functionalization of nanoparticles (NPs) with different biomolecules, such as antibodies, aptamers, and enzymes, facilitates their sensitive detection and targeted delivery to BC cells. Nanotechnology is rapidly growing with many types of NPs that are exploited to address various issues, such as tumor imaging, as carriers for chemotherapy drug delivery to minimize side effects, or improving drug utilization in intravesical instillation.

Synthetic Nanomaterials Against BC

Some NPs have been reported to be potential nanotherapeutic agents of BC, for example, cuprous oxide NPs[54] and gold NPs derived from Abies spectabilis extract.[55] The majority of researchers add various elements to nanomaterials to increase their properties. Titanate nanotubes (TNTs) with good biocompatibility and tumor cytotoxicity can improve the efficacy of radiotherapy, whereas quercetin (Qc) promotes tumor cell apoptosis by activating the mitochondrial pathway.[56] Alban *et al*[57] incorporated Qc in zinc titanate nanotubes (ZnTNT) to form a new tubular nanostructure. ZnTNT has enhanced thermal stability and increased the bioavailability of Qc. Cytotoxicity results showed that Qc-coated TNT nanomaterials significantly decreased BC cancer cell viability and increased the effectiveness of *in vitro* radiotherapy. Li *et al*[58] utilized fluorinated polyethyleneimine (F-PEI) to deliver an active venom peptide, polybia-mastoparan I (MPI). MPI is an antibiotic peptide derived from the venom of the social wasp *Polybia paulista*. F-PEI and MPI can self-assemble to form stable NPs after mixing. The combination of F-PEI and MPI achieved high tumor growth inhibition efficacy, even superior to the classic chemotherapeutic drug mitomycin C. In addition, a study reported the use of poly (amidoamine) to modify mesoporous silica NPs (MSNPs) to increase their mucoadhesion and deliver doxorubicin (DOX) for BC intravesical treatments.[59]

Nanomaterials for Improving Recognition Specificity and Killing Ability for BC

The bladder connects with the outside world through the urethra, which allows the implementation of intravesical instillation. This mode of administration is far safer, more convenient, and has fewer side effects than systemic administration.[60,61] Nonetheless, some challenges are also noted. Various physiological barriers of the bladder impede the effective penetration of drugs, and the retention time of chemotherapeutic drugs in the bladder cavity is limited.[61,62] Thus, increasing the mucoadhesion or tumor-targeting specificity of the materials is conducive to treatment.

Quite a few nanomaterials have excellent mucoadhesion capabilities. Researchers further endow them with the capabilities to respond to tumor- or microenvironment-related factors, thereby improving BC detection and treatment. For instance, *survivin*, a member of the apoptosis inhibitory protein family, can serve as a specific marker for BC.[63] For sensitive detection of BC margins in surgery, nanoprobes (AuNP-MB@R11) containing molecular beacon (MB) corresponding to survivin and R11 peptide-linked spherical nucleic acids (SNAs) were synthesized.[64] Researchers first constructed SNAs composed of AuNPs and MB to quantify survivin mRNA.[65] Then, due to the super BC targeting and cellular uptake potency of the poly (11)-arginine cell-penetrating peptide (R11), they further attached R11 peptides to SNAs to form AuNP-MB@R11 NPs. The mechanism by which these nanomaterial works has been described. Specifically, when survivin mRNA is absent, MB is in the off state, and the fluorophore Cy5 is close to the surface of AuNP, which results in fluorescence quenching by fluorescence resonance energy transfer (FRET). Conversely, when survivin mRNAs bind to the MBs, the conformation of MBs changes, and Cy5 moves away from the AuNP surface, releasing fluorescent signals. A cancerous fluorescence margin is observed. Thus, AuNP-MB@R11 can be regarded as an intraoperative molecular imaging technique to achieve complete tumor resection and improve recurrence-free survival.[64] In addition, chitosan-based nanomedicine that delivers gambogic acid prodrug in response to GSH and ROS signaling has also been reported.[66] Cationic chitosan is a well-known mucoadhesive biomolecule that significantly enhances drug adhesion to and penetration of the bladder wall. This system was designed to first respond to GSH to release the prodrug of gambogic acid. The prodrug is then activated by ROS and converted to gambogic acid, a known anticancer drug extracted from the tree *Garcinia hanburyi*, to inhibit BC. The dual response of GSH and ROS effectively reduces the damage to the urethra and normal urothelium.

In addition to NPs that respond to tumor- or microenvironment-related factors to release drugs, some nanomaterials were employed to deliver siRNAs targeting oncogenes of BC, such as natural halloysite nanotubes (HNTs). Researchers used vacuum impregnation methods to make the lumen of the halloysites (~500 nm) with a positive charge fill of negatively charged siRNAs that can downregulate the receptor-interacting protein kinase 4 (*RIPK4*). HNTs/siRNA complexes can pass through various physiological barriers and eventually concentrate in the tumor tissues without inducing the activation of the innate immune response.[67] Liang *et al*[68] coupled chitosan-hyaluronic acid dialdehyde NPs with antibodies against *CD44* to deliver siRNA targeting the oncogene *Bcl2*. This NP system significantly downregulated *Bcl2* expression and effectively inhibited BC progression.

Other studies link nanomaterials with biological enzymes to enhance anticancer effects using substances specific to urine or tumors. Hortelão *et al*[69] developed urease-driven nanomotors based on MSNPs. The outer surface of MSNPs is coupled with urease, PEG molecules, and fibroblast growth factor receptor 3 (*FGFR3*) antibodies. *FGFR3* antibodies on the nanomotors interact with the tumor antigens in BC 3D spheroids and lead to more efficient nanomotor internalization and blockade of the

fibroblast growth factor signaling pathway to inhibit cell growth and proliferation. Urea in urine is biocatalytically converted to ammonia and carbon dioxide by urease, and increased concentrations of ammonia near the nanomotors are associated with greater cytotoxicity. In addition, a study combined sonodynamic therapy (SDT) with NPs to develop a transmucosal oxygen-self-production SDT platform for intravesical instillation of BC.[70] A commonly used sonosensitizer meso-tetra (4-carboxyphenyl)porphine (TCPP) was conjugated with catalase (CAT), which were then mixed with FCS to form CAT-TCPP/FCS NPs. CAT-TCPP/FCS NPs are triggered by non-invasive ultrasound, and then facilitate actin contraction and cytoskeleton rearrangement, resulting in transient opening of tight junctions and enhancing the intratumoral penetration of NPs. Then, excessive endogenous H_2O_2 in the tumor is catalyzed by CAT to generate O_2 to relieve tumor tissue hypoxia. The nanosystem had favorable biosafety and therapeutic efficacy by suppressing BC growth through the ROS-mediated apoptosis pathway. Beyond these, Sun *et al*[71] developed a photoactivated H2 nanogenerator composed of FCS, a catalyst for H_2 production, and the chemotherapeutic drug gemcitabine. After intravesical instillation, NPs triggered the *in situ* generation of H_2 upon 660 nm laser irradiation. Hydrogen combined with gemcitabine promoted the phosphorylation of the mitochondrial function-related factor *VDAC1*, which repressed mitochondrial function and ATP synthesis.[72,73] Therefore, drug efflux mediated by the P-gp protein belonging to the ATP binding cassette transporter superfamily is reduced due to a lack of energy from ATP hydrolysis.[74,75] The photoactivated H_2 nanogenerator exhibited excellent transmucosal and cellular uptake abilities and effectively suppressed chemoresistance.

Multimodal Therapies for BC

To better detect and eliminate BC, more sophisticated nanomaterials have also been designed. Tao *et al*[76] combined magnetic resonance imaging (MRI), chemotherapy, PTT, and nanotechnology to prepare folate-modified vincristine (VCR)-loaded polydopamine-coated Fe_3O_4 superparticles (Fe_3O_4@PDA-VCR-FA SPs). Fe_3O_4 NPs can be used as MRI contrast agents given their magnetic properties, and photothermal reagents that absorb NIR light and then convert it into heat to kill cancer cells. PDA was used as the shell of SPs, which not only reduced the toxicity but also improved the photothermal conversion ability and extended the half-life of SPs in blood. The chemotherapeutic drug VCR was loaded into the PDA shell. Folic acid (FA) modification greatly improved the bioavailability of nanomaterials. When the Fe_3O_4@PDA-VCR-FA SPs reached the tumor, the acidic tumor environment and the externally administered NIR laser-induced the release of VCR, effectively reducing the systemic side effects while completing chemotherapy. In another study, researchers conjugated upconversion NPs (UCNPs) and gold nanorods (AuNRs), and then coupled them with epidermal growth factor receptor (EGFR) antibodies to specifically target BC cells. Due to the interaction of antigen and antibody, UCNP-AuNR nanoclusters can be uniformly bound to EGFR-positive cell membranes, thus ensuring the therapeutic effect of intravesical instillation. Under NIR excitation, UCNPs capture low-energy photons and upconvert them to higher energy photons in the visible region via FRET, producing high-contrast images for BC detection *in situ*. After irradiation of UCNP-AuNR-treated cells, the cell membrane was ruptured, and low-dose cisplatin was sufficient to kill them. This is an example of a potential clinical application of UCNP-AuNR nanoclusters to detect and diagnose BC by intravesical instillation.[77]

Compared with nucleic acid-based therapy and genetically modified T-cell immunotherapy, nanomaterials appear to be more fully exploited for BC diagnosis and treatment. One of the reasons is that nanomaterials have good biocompatibility and drug loading capability, and are easy to be modified according to individual purpose. Another reason is that nanomaterials can treat bladder diseases through intravesical instillation, avoiding the burden on the liver and kidney caused by systemic therapy of nanomaterials. However, the clinical trials of nanomaterials in BC are few, and their biological safety remains to be evaluated. Even if nanomaterials are still far from widespread clinical application, their functional diversity and good plasticity increase their potential for application in BC.

Conclusion

In recent decades, many attempts have been made to develop new techniques for the early detection and specific killing of BC. Herein, we emphasize the key progress of synthetic biology in BC and discuss the implications. Synthetic biology has been extensively applied in various aspects of BC to improve the sensitivity and effectiveness of BC diagnosis and treatment. However, more in-depth research needs to be performed, such as programming bacteria to diagnose and treat BC or developing appropriate T-cell editing strategies to improve killing efficiency and safety. Synthetic biology is a promising clinical tool in the treatment of BC.

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Conflicts of interest

None.

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