



## Recent development on synthetic biological devices treating bladder cancer

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### ABSTRACT

Synthetic biology is an emerging field focusing on engineering genetic devices and biomolecular systems for a variety of applications from basic biology to biotechnology and medicine. Thanks to the tremendous advances in genomics and the chemical synthesis of DNA in the past decade, scientists are now able to engineer genetic devices and circuits for cancer research and intervention, which offer promising therapeutic strategies for cancer treatment. In this article, we provide a systemic review on recent development achieved by the synthetic biologists, oncologists and clinicians of one National “973” Plan. We expand the synthetic biology toolkits involving DNA, RNA and protein bio-parts to explore various issues in cancer research, such as elucidation of mechanisms and pathways, creation of new diagnostic tools and invention of novel therapeutic approaches. We claimed that the Chinese synthetic biologists are promoting the basic research productions of tumor synthetic biology into the clinic.

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### Contents

1. Introduction .....	216
2. The progress of tumor synthetic biology in “973” Plan .....	217
2.1. BCa targets identified by genetic switches .....	217
2.2. Cancer specific promoter for driving anti-target devices .....	217
2.3. Logical and gates for identification of bladder cancer .....	217
2.4. Redirecting oncogenic signaling to an antioncogenic pathway .....	218
3. Perspective and future works .....	218
References .....	220

### 1. Introduction

Urinary bladder cancer (BCa) is the most common urinary tract malignancy with high recurrence and mortality rates all around the

world. Each year, bladder cancer is diagnosed in about 80500 patients in the China and in more than 429793 patients worldwide, making it the 5th most common cancer in men and the 9th most common cancer in both sex [1,2]. Although the cancer therapy has achieved some improvements, the trends in BCa incidence and mortality has not been decreased significantly in recent years which indicate that the developments of therapies for BCa were not ideal. What's more, limited specificity and efficiency were shown in current therapies for BCa, such as chemotherapy, surgery and radiation [3–5]. An innovative and precise treatment is crucially required in this issue and further studies will be needed to pinpoint the concrete pathogenesis of BCa. Since the unparalleled progresses in cancer

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genetics and genomics have been achieved, precision medicine captured our great attention. The application of systems biology and panomic analysis to analyze the cause of an individual patient's disease plays a vital role in precision medicine. A comprehensive analysis of genetic alterations in BCa was performed in our former studies which show frequent mutations of chromatin remodeling genes and frequent alterations in genes involved in sister chromatid cohesion and segregation [6,7]. Moreover, we screened somatic mutations of BCa and found that mutation of the androgen receptor gene is not associated with BCa and mutant TERT promoter may serve as potential markers for the differential diagnosis and surveillance of BCa [8,9]. Together, these data reveal the necessity of epigenomics research in the future cancer studies.

As mentioned above, current situation calls for the need to develop a novel powerful therapy which could fulfill precision medicine. Although still in its infancy, synthetic biology approach has been used in biomedical field. Synthetic medicine is an emerging scientific field at the interface of synthetic biology and medicine that creates functional devices for clinical medical uses. The main goal of medical synthetic biology is to develop technologies that are designed to assist in diagnosis or reverse disease states. They are believed to reach the clinical setting in the near future. Just in recently, an increasing number of original functional circuits are designed and created to expand the tools available for therapeutic and research applications. From bacterial hosts to mammalian cells, synthetic biologists have opened a door for updating our knowledge of cellular networks and stimulating the development of novel therapeutic approaches, especially in cancers [10,11]. Severe side effect and large scale damage of noncancerous cells were usually happened in conventional therapies, such as chemotherapy and radiation [12]. Benefited from the versatile cancer-killing switches and engineered cells, precision and efficacy in targeting and killing cancer cell have been greatly improved. Moreover, the cancer cellular behavior can be rewired to produce new behaviors [13]. All of these studies have enabled more and more novel strategies to treat cancers.

During recent years, tumor synthetic biology has already captured a huge attention from China's government and a series of investment on this topic has significantly increased in the coming years. Project "Bladder Cancer Intervention with Engineered Bio-devices project", one of the National Key Basic Research Plan (the National "973" Plan), has been launched to keep Chinese synthetic biologists in the forefront of this emerging field for the transformation of tumor synthetic biology into the clinic. In this review, we focus the recent development on synthetic biological devices treating bladder cancer in the National "973" Plan. Throughout, we discuss the progress and perspectives of tumor synthetic biology in China.

## 2. The progress of tumor synthetic biology in "973" Plan

### 2.1. BCa targets identified by genetic switches

Although various useful prediction models of BCa have been reported in recent years, there is still a requirement for more novel biomarkers for improving the specificity and reliability of prediction tools for BCa [4]. Accumulating pioneering studies suggested that lncRNAs are involved in development in different cancers [14]. Further comprehensive mechanism between lncRNA and BCa was discovered in continuance [15]. Thus, the identification of BCa-associated lncRNAs may provide an opportunity to better understand the mechanism network of cancer pathogenesis in BCa for future targeted treatment. In contrast to the traditional researches, we applied a novel strategy to identify the functional BCa-associated lncRNAs. Based on the engineering principle of synthetic biology, we constructed tetracycline-inducible RNAi devices

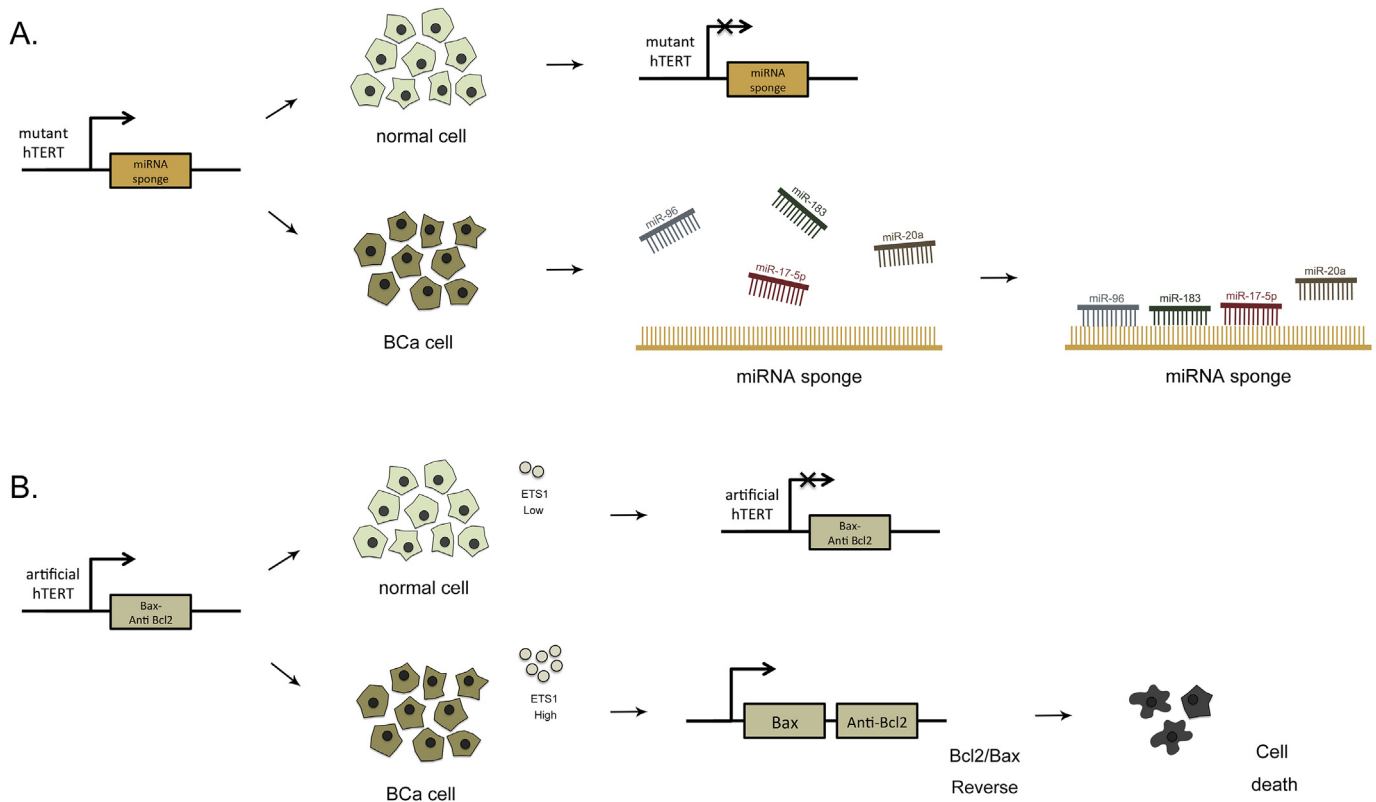
targeting BCa-associated genes. Benefited from the tet-RNAi device, we can regulate the expression of the target genes in doxycycline dosage-dependent manner for the verification of its function in BCa. In order to prove the utility of these devices, we have constructed artificial miRNAs that target lncRNAs or protein-coding mRNAs. These devices could induce anti-cancer effects by silencing targeted protein-coding genes or non-coding genes, accordingly [16]. Then tet-shRNA/miRNA were constructed to target and silence star-oncogene. We found that both the tet-shRNA [17] and tet-miRNA [18] could effectively silence the target genes and inhibit related malignant phenotypes of BCa, such as induction of BCa cell apoptosis, inhibition of BCa cell proliferation and suppression migration. To further expand on this issue, we evaluated the function of BCa-related lncRNAs by the tet-RNAi devices after series of preliminary function tests [19,20]. The lncRNA-associated malignant phenotypes of BCa can be quantitatively regulated by controlling the relative expression of target lncRNAs. To improve the RNAi efficiency in target regulation, we construct the tetracycline-inducible double shRNAs targeting multi-lncRNAs [21]. Together, lncRNA PVT1, HIF1A-AS2 and CCAT2 might serve as oncogenes in BCa and might be employed as potential therapeutic targets for BCa by the further identification of the tet-RNAi device. Through the useful tet-RNAi devices, the target genes could be precisely regulated in a dosage-dependent manner. So we can accurately identify novel and useful biomarkers for precision cancer treatment.

### 2.2. Cancer specific promoter for driving anti-target devices

Tumor specific promoters are specifically activated in tumor cells, such as AFP promoter in hepatocellular carcinoma, PSA promoter in prostate cancers and so on. All these promoters have presented an novel and effective approach to improve the specificity of cancer therapy. In the previous work [8], we found that the somatic mutations in TERT promoter can be detected in 55.6% of the BCa, and that these mutations can up-regulate the expression of hTERT and enhanced related tumor-specific feature in BCa. Also the mutant hTERT promoter has a higher transcriptional activity in BCa and affects patient survival and disease recurrence [22]. Hence, mutant hTERT promoter can be employed as useful BCa-specific element in anti-BCa module. Based on these studies, we constructed synthetic miRNA sponges (Fig. 1A) which could selectively suppress the expression of oncogenic miRNAs driven by mutant hTERT promoter [23]. This miRNA sponges not only inhibited proliferation and migration but also induced apoptosis by suppression of miR-17-5p, miR-20a, miR-96, and miR-183 in BCa cell while human fiber cells were nearly not affected by the miRNA sponge. It was manifested that the synthetic miRNA sponges driven by mutant hTERT promoter had tumor-specific effects on BCa cells. Based on the wild-type hTERT promoter sequence, we constructed the artificial hTERT promoter that has a significantly improved driven efficiency and still retains a high cancer-specificity in BCa. The driven efficiency of artificial hTERT promoter was associated with ETS-1. Furthermore, the Bax-Anti Bcl2 combination module (Fig. 1B) driven by artificial hTERT promoter could specifically and effectively inhibit malignant phenotypes of BCa by reversing the ratio of Bcl2/Bax [24]. Both mutant hTERT promoter and artificial hTERT promoter could robustly and specifically induce the expression of synthetic modules, thus presenting a notable strategy for tumor-targeting therapies.

### 2.3. Logical and gates for identification of bladder cancer

The CRISPR (clustered regularly interspersed with short palindromic repeat)/Cas is derived from the immune systems of bacteria



**Fig. 1.** Design and construction of anti-target devices driven by cancer specific promoter. A. The schematic representations of the synthetic miRNA sponges driven by mutant hTERT promoter. The suppression of oncogenic miRNAs was only detected in BCa cells while normal cells were nearly not affected. B. The schematic representations of the artificial hTERT promoter-Bax-Anti Bcl2 combination module. In the BCa cell, the expression level of transcription factor ETS-1 is high and thus the artificial promoter is active. Oppositely, ETS-1 is absent and the artificial promoter is inactive in the normal cells.

and archaea. It uses RNA with specificity to direct Cas protein in the modification of the target site sequence. Until recently, scientists have begun to use this system to generate targeted mutations in the genomes of animals. To date, researchers have successfully applied the CRISPR/Cas system to human, mouse, zebrafish, silkworm, *Drosophila*, yeast, *Arabidopsis thaliana* and rice. It is also used to explore the mechanisms of a series of human diseases such as cancer, AIDS, and inherited disorders [25]. We reported an article in Nature Communications, in which our group constructed an AND gate genetic circuit (Fig. 2A) based on the CRISPR-Cas9 system [26]. The genetic circuit integrates the information from the two promoters as the input, and the output signal is activated only when the two inputs are activated in the test cell lines (Fig. 2B). Using luciferase reporter gene as a genetic output (Fig. 2C), we confirmed that compared to human telomerase reverse transcriptase (hTERT) - Renilla fluorescence enzyme (Renilla luciferase) constructs, this circuit can specifically detect bladder cancer cells and significantly improve the luciferase expression. In addition, hbax, p21 and E-cadherin were used to replace the luciferase reporter gene as the output gene (Fig. 2C), and the results showed that the malignant phenotypes of bladder cancer cells can be inhibited effectively by regulating the corresponding genes. This method provides a synthetic biological platform for the *in vitro* targeting and control of bladder cancer cells.

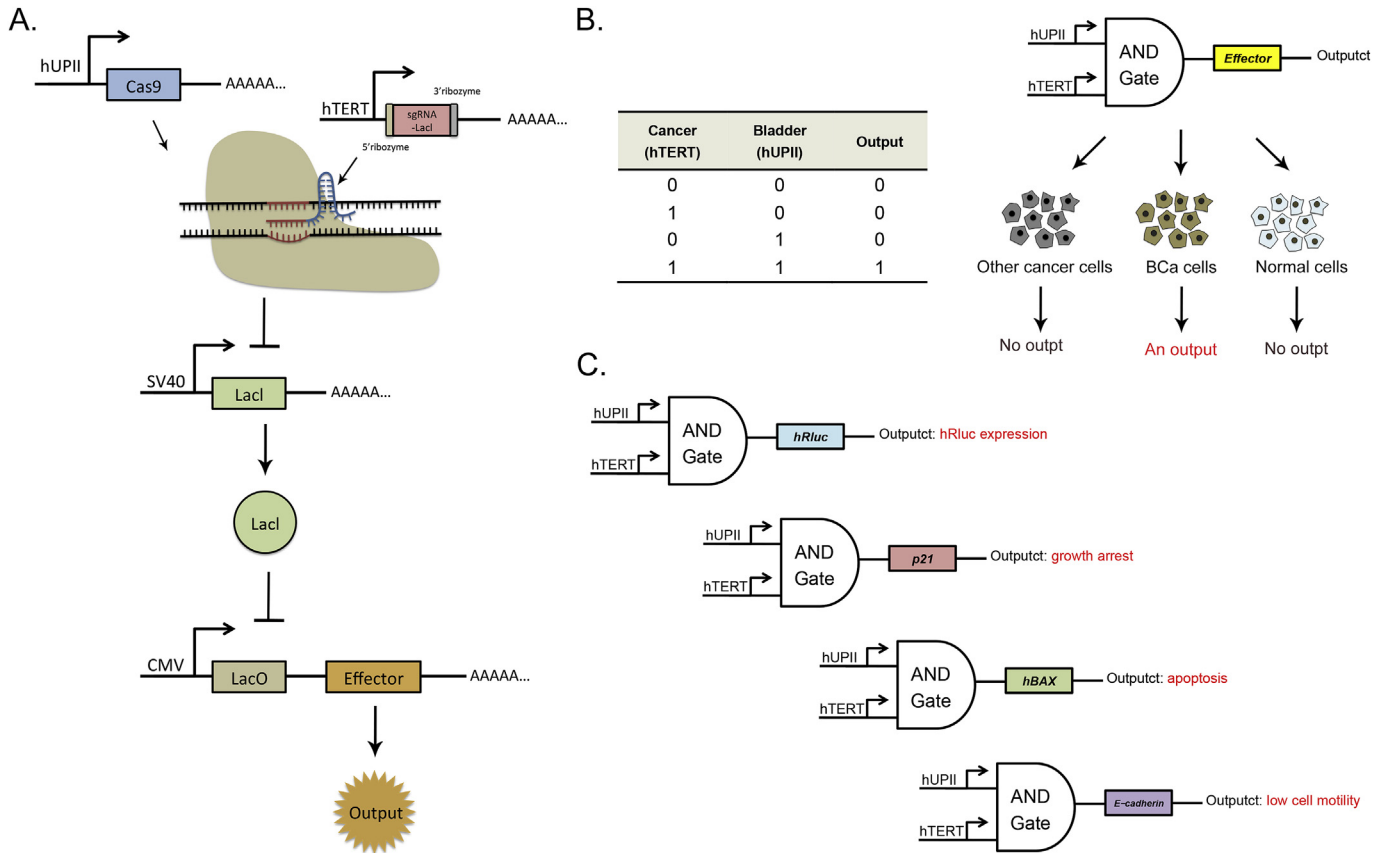
#### 2.4. Redirecting oncogenic signaling to an antioncogenic pathway

We also reconstructed the CRISPR-Cas9 system by combining the riboswitch recognizing specific biological signal into the sgRNA scaffold [27] (Fig. 3). These newly-engineered genetically-encoded

devices carry and distribute external or internal input signal currents to produce designated output responses, thus functioning as “signal-conductors” that build interconnection networks for signal redirecting applications. In the molecular design of one such device, the riboswitch responds to one specific small molecule or protein signal while the binding motif of sgRNA targets the gene encoding another signal and interferes with transcription, either positively or negatively. A different transcriptional response will result in a novel cell fate decision. This work supports the idea that cellular signals can be connected in novel combinations by the CRISPR-Cas9-based signal-conductors, which suggests a new dimension to the ability of CRISPRs to edit complex cellular processes. We used these signal-conductors to build a set of transcriptional logic gates which integrate endogenous signals as the inputs and control the expression of one cellular gene as the output. We also show that the signal-conductors can be used to establish synthetic cross-talks between different signaling pathways in bladder cancer cells. Furthermore, we demonstrate that these devices can act as a master redirector of oncogenic signals and thus reprogram the fate of bladder cancer cells. By rewiring gene networks we can shed light on the treatment of bladder cancer that occurs when native networks are damaged. These new devices may facilitate a new approach to selectively kill cancer cells in which the oncogenic signals are dysregulated, because they can link the oncogenic signals to the activation of anti-oncogenic signals.

### 3. Perspective and future works

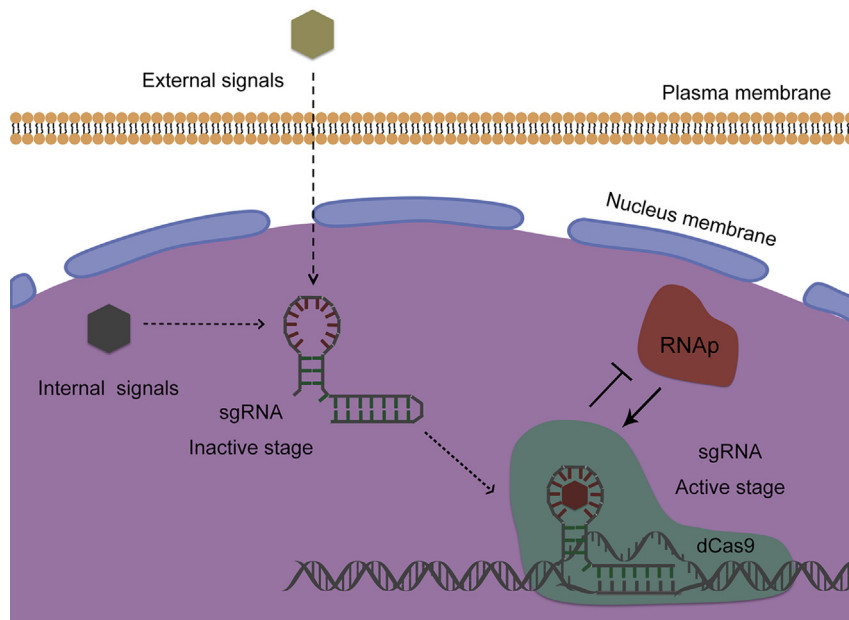
The above proposed synthetic biology strategy will provide a novel therapeutic approach with high specificity and efficiency



**Fig. 2.** Design and construction of the AND gate genetic circuits. A. The designed circuit is based on the CRISPR/Cas9 system and used the hTERT and hUP II promoters as the two inputs. B. General principle of the AND gate circuit in identification of bladder cancer cells. The circuit only produces a high output in bladder cancer cells. C. The schematic representations of the synthetic circuits. The output genes were hRluc, hBAX, p21 and E-cadherin, respectively.

characteristics. Such success will make up for defects of traditional bladder cancer treatments. Developing artificial biological systems to control the malignant phenotypes of tumor cells also can answer

many fundamental scientific problems, and greatly promote the development in the field of cancer therapy. In the future works, we will test the efficiency and specificity of these synthetic devices in



**Fig. 3.** General illustration of the CRISPR–Cas9-based signal-conductor. The device was composed of two parts: a sensing module, made of a riboswitch; and a sgRNA module, which was exposed due to a conformational change after the signal bound to the aptamer. It allowed highly responsive, dose-dependent and dynamic control of mammalian gene expression.

the treatment of mice and patients suffering from bladder cancer, further optimize specific killing effects on bladder cancer and extend this approach to other types of tumors. Even though synthetic circuit therapy is making its way to treat a number of human cancers since the recent years of basic experimental research, the safe and effective use of gene delivery system remains a challenge. The future works should also improve the gene delivery vectors (e.g. the nanoparticles) and use the principles of controlled release strategies to increase the biosafety.

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