Turing miRNA Into Infinite Coordination Supermolecule: A General and Enabling Nanoengineering Strategy for Resurrecting Nuclear Acid Therapeutics

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Abstract

Background

Clinical translation of therapeutic nuclear acid, particularly those targeting tumor progression, has been hampered by the intrinsic weaknesses of nuclear acid therapeutic including poor systemic stability, rapid clearance, low membrane permeability and lack of targeting ability. Small nuclear acid engineered into carrier-free nanodrugs with structural stability and disease targeting may be viable to overcome pharmaceutical obstacles of nuclear acid.

Methods

A general method through a mild and simple chemistry was established to convert therapeutic miRNA into an infinite Auric-sulfhydryl coordination supramolecular miRNA termed lacsRNA with near-spherical nanostructure, high colloid as well as anti-hydrolysis stability and low macrophage uptakes.

Results

lacsRNA presented the increased half-life period in circulation and accumulation at tumor sites in comparison to normal miRNA. Moreover, lacs-miR-30c showed no toxicity of viscera and sanguis system in the 5-time injection dosage of the treatment. More importantly, lacs-miR-30c potently suppressed the Wnt signaling pathway *in vitro* and *in vivo*, and effectively sensitized both potency of 5-Fu in PDX model of colon cancer and Anti-PD1 in B16F10 homograft model of melanoma.

Conclusion

Collectively, this work amply confirmed the design of lacsRNA as a general and viable strategy of nano-pharmaceutic to concert flimsy therapeutic miRNA into potential drugs. Considering from a broader perspective, the miRNA-initiated infinite coordination self-assembly strategy has distinct advantages in resurrecting nuclear acid therapeutics, probably bringing new inspiration to RNA-derived therapeutics of a great variety of human diseases including cancer.

Introduction

MicroRNAs (miRNA), a series of 22 nucleotide-long and noncoding RNA molecules, regulate gene expression and a range of biological functions involving in the pathogenesis of a wide variety of diseases in especial of cancer [1]. In the cytoplasm, miRNAs recognize a complimentary mRNA sequence, and subsequently induce its post-translation degradation and silencing, offering great promise to create novel therapeutic approaches at transcriptional level towards down-regulating abnormally elevated pathogenesis-related proteins [2, 3]. Although a growing number of miRNAs modulating disease

processes have been identifying, their clinical translation, particularly those targeting tumor progression, has been hampered by the intrinsic weaknesses of nuclear acid therapeutic including poor systemic stability, rapid clearance, low membrane permeability and lack of targeting ability [1, 4].

To alleviate these technical hurdles, various elaborate methods for miRNA modification and/or delivery have been developed, and significant progress has been made in ways of improving pharmacological potentials of miRNAs therapeutics [1]. Though chemically modified miRNAs possess the resistant against nuclease and the subsequent prolonged half-life in the bloodstream, the off-target effects severely limit their clinical application presumably because of the broad but excrescent functionality of miRNA in the healthy organs and tissues [5, 6]. Besides, as for miRNAs delivery vehicles, the frequently-used lipids, cationic polymers and biodegradable polymers always suffered from the rapid clearance by reticuloendothelial system (RES), in which liver and spleen macrophages eliminate these exogenous particles at a great lick from the circulation system [7, 8], resulting in the bereft or depressed potency of miRNAs therapeutics. Conceptually, novel and clinically viable strategies are needed to advance the clinical transformation of miRNAs.

Nanotechnology holds great promise in overcoming pharmacological weaknesses of miRNA [9, 10]. Nanocarrier-mediated oligonucleotide delivery is capable of protecting the cargo from nuclease degradation during the circulation, and promoting oligonucleotide internalizing into cytoplasm *via* endocytosis that can escape endosomal degradation [11–13]. In fact, nanoparticle-based drug delivery systems are particularly attractive in the treatment of solid tumors, as nanoparticles are capable of active crossing endothelial cells into the interstitial space of the neoplasm, thereby resulting in the preferential accumulation at tumor sites [14–16]. Despite increasing success in the development of tumor-targeting nanocarriers [17–21], the overwhelming majority of nano-medicine suffered from time/cost-consuming construction, low cargo loading and improper disruption in circulation [22, 23]. Therefore, it remains a challenge to explore miRNA-derived nanotherapeutics that simultaneously possess simple synthesis, high stability, satisfying loading and excellent performance.

Carrier-free nanodrugs provided an intriguing strategy to develop nanomedicine, by which drug themselves were assembled into well-defined nanostructures through self-assembly and/or coordination [24, 25]. Infinite coordination polymers (ICP) is an emerging class of carrier-free nanodrug system, in which drugs connect with ions directly *via* coordination bonds [26, 27], yielding a series of advantages including adjustable high drug loading, compositional tunability, mild preparing condition, and infinitely controllable extension in spatial dimension [26, 27]. As a consequence, we hypothesized that miRNA-derived ICP can not only overcome the pharmacological weaknesses of nuclear acid therapeutics, but also possess excellent pharmaceutical superiority towards the optimized potency.

Herein, we developed a general method to convert therapeutic miRNA into a stable and bioavailable infinite **Auric-sulfhydryl** coordination supramolecular **miRNA** (lacsRNA) by a mild and simple chemistry route. In this case, auric-mercapto-miRNA precursors can self-assembled into a spherical nanostructure driven by aurophilicity (Figure 1A). As a proof of concept, miR-30c, a member of miR-30s family that

specifically target Bcl9 in cancer[28], was used to synthesized lacsRNA (Figure 1A). As a result, lacs-miR-30c overcome the pharmacological obstacles of miRNA and potently suppressed the Wnt signaling pathway *in vitro* and *in vivo*, but more than that, it effectively potentiated chemotherapy and immunotherapy *in vivo*. This work amply confirmed the design of lacsRNA as a general and viable strategy of nano-pharmaceutic to concert therapeutic miRNA into potential drugs, thereby reinvigorating efforts for discovering nuclear acid therapeutics in a great variety of human diseases including cancer.

Results

Design and Synthesis of lacsRNA

For the construction of infinite Auric-sulfhydryl coordination polymeric miRNA (lacsRNA), an extra sulfhydryl was introduced to the 5' terminus of miRNA, and Au^{3+} in chloroauric acid was used to conjugate with the thiol to form a polymeric RNA-Au(I) compound termed $[\text{RNA-S-Au}^+]_n$ through infinite extension in 2D (Figure 1A), where Au^{1+} ions bridge miRNA-SH by a bivalent -S- Au^+ -S- coordination[19, 29–31]. Subsequently, driven by the aurophilicity among Au(I), $[\text{RNA-S-Au}^+]_n$ can self-assemble into a near-spherical architecture (lacsRNA) at small nano scale [18, 30], supporting by the transmission electron microscope (TEM) image of lacsRNA (Figure 1B) and hydrodynamic diameter of lacsRNA around 38.7nm with an acceptable polymey disperse index of 0.265 measured by dynamic light scattering analysis (Figure 1C). Additionally, the surface charge of lacsRNA was -11.1 mV (Figure 1D), and this electronegativity implied that lacsRNA would resist coronin and get a long half-life during circulation [32]. Moreover, UV-Vis (Figure S1) and FT-IR (Figure 1E) spectroscopy further confirmed the successful assembly of lacsRNA as evidenced by characteristic absorbance given by Mercapto modified RNA. The valence states of S and Au in lacsRNA were explore by X-ray photoelectron spectroscopy (XPS) analysis, in which S element presented +4 valence in line with the expected molecular composition in $[\text{RNA-S-Au}^+]_n$ (Figure 1F). Of note, the electronic energy signals given by Au in lacsRNA were low than the base electronic energy of Au atoms (Figure 1G), presumably because of the electron migration from S to Au after conjugation, thereby further proving the construction of the infinite Auric-sulfhydryl coordination polymeric miRNA. Collectively, these results demonstrated the successful conversion from miRNA to a near-spherical polymeric miRNA nanoparticle through a simple and mild “one-pot” chemistry.

Physicochemical and pharmaceutical properties of lacsRNA

As our design, lacsRNA should be endowed with the resistance against endonuclease, because of the steric hindrance of near-spherical nanostructure against enzyme recognition and binding [33, 34]. To explore it, nude miRNA and its corresponding lacsRNA were incubated in sterile PBS buffer containing 20% foetal bovine serum (FBS), and the residue RNA were semi-quantified by agarose gel electrophoresis (Figure 2A). In this case, lacsRNA obviously prolonged the half-time of RNA from $3.2 \pm 0.4\text{h}$ to over 24 hours (Figure 2B)., indicating that lacsRNA strategy can overcome the poor systemic stability -the major

pharmaceutical obstacle- of nuclear acid therapeutics. Next, to explore the cellular internalization of lacsRNA, Sulfo-Cyanine3 (Cy3) was 3'-terminally conjugated for the cellular uptakes examination by flow cytometry analysis. As shown in Figures 2C and D, lacsRNA (91.6%) showed much more internalization into B16F10 melanoma cells than miRNA without transfection (39.4%) after 6h incubation. What's even more amazing is lacsRNA prevented the macrophages uptakes of miRNA (Figures 2C and D), providing a highly favorable profile for circulation. This result compelled us to explore the colloidal stability of lacsRNA that is another important influencing factor for blood circulation. As expected, both in pH 7.4 and pH 6.5, the incubation in PBS including 20% serum cannot alter the hydrodynamic diameter of lacsRNA (Figure 2E), indicative of low coronin and high colloidal stability. As a result, compared to miRNA alone, lacsRNA significantly improved the circulation time in blood after systematic injection quantified by the fluorescence signal from the labeled Cy3 fluorescein in miRNA (Figure 2F).

According to the enhanced permeability and retention (EPR) effect of nanoparticle, satisfactory circulation time always resulted in the promotional tumor accumulation. To verify it, we determined the biodistribution of lacsRNA in B16F10 homograft model of melanoma by inductively coupled plasma mass spectrometry (ICP-MS), which was used to quantify ^{197}Au concentrations in tissues. After intravenous injection of 2 mg/Kg lacsRNA, a time-dependent tendency for tumor accumulation can be found in the biodistribution results (Figure 1G) and calculating the accumulation ratios of tumor versus normal organs (Figure 1H). To visually examine the different tumor accumulation between miRNA and lacsRNA, Cy3-labeled samples were intravenously injected into the tumor-bearing mice. At 6h post-injection, ex vivo fluorescence imaging and quantification (Figures 2I and J) revealed over 4-times tumor accumulation in Cys-lacsRNA-treated mice in comparison to Cys-miRNA-treated mice. Taken together, these results demonstrated that lacsRNA strategy overcome the intrinsic weaknesses of nuclear acid therapeutic including poor systemic stability, rapid clearance, low membrane permeability and lack of tumor targeting.

The safety evaluation of lacsRNA *in vivo*

The superior performance of lacsRNA in pharmaceutical properties further compelled us to study its safety *in vivo*. To assess the safety of lacs-miR-30C, healthy C57/BL6 mice were intravenously injected with normal saline (Control) or lacs-miR-30C respectively every other day at a dosage of 10 mg/kg, which was 5-times the therapeutic dose (n = 5 per group). After 9 days of administration, no hepatotoxicity can be found as evidenced by the no difference of aspartate transaminase (ALT), alanine aminotransferase (AST) and pathological section of liver between Control and lacsRNA group (Figure 3A). Besides, the 9-day administration have no effect on the blood urea nitrogen (BUN), creatinine (CREA) and Hematoxylin&Eosin (H&E) staining slices of kidney (Figure 3B), indicative of the hardly any nephrotoxicity. In addition, no hemolysis, myelosuppression, anemia, leukopenia and thrombopenia was found after lacs-miR-30C treatment (Figure 3C). Moreover, compared with control group, lacs-miR-30C treatment had almost no effect on body weight (Figure 3C, D), which further supported the highly favorable biosafety profile of lacs-miR-30C. Additionally, no pathological morphological changes can be

found in the heart, lung, and spleen at lacs-miR-30C-treated mice. Collectively, these data illustrated that lacs-miR-30C is avirulent enough for clinical translation.

lacsRNA Potently Suppressed Wnt/ β -catenin Pathway

MiR-30c is known to inhibit oncogenic Wnt/ β -catenin activation through suppressing the expression of Bcl9 [21]. To comparatively investigate the potency of lacs-miR-30c and commercial miR-30c, B16F10 cell line- a kind of malignant and Wnt-hyperactive melanoma- was used to challenge their action via a 24-hour incubation at the concentration of 50 nM. As expected, lacs-miR-30c enhanced the accumulation of miR-30C in cells and suppressed the expression of Bcl9 potently, whereas carrier-free miR-30C showed no difference between PBS-mock-treated control (Figures 4A and B). At a transcriptional level, lacs-miR-30C triggered 360 differentially expressed genes after 24-hour incubation on B16F10 cells as evidenced by RNA sequencing and subsequent clustering analysis (n=3) (Figure 4C). Gene set enrichment analysis (GSEA) exposed that suppression signatures were enriched in β -catenin gene sets in lacs-miR-30C-treated cells compared to the mock-treated cells (Figure 4D-F). Meanwhile, there was also a remarkably enrichment of suppression features presented in Wnt signaling pathway (Figure 4G-I). As a consequence, lacs-miR-30C induced the cell cycle arrest of cancer cells, as reflected in GSEA analysis in cell cycle, cell cycle checkpoints and cell cycle mitotic (Figures 4J and S2). This result was proved again by the significantly decreased percentage of S phase of cells measured by the PI-staining-derived cell cycle analysis (Figures 4K and S3). Together, above results demonstrated that lacsRNA strategy resurrected the action of miR-30c and potently suppressed Wnt/ β -catenin pathway *in vitro*.

lacsRNA potently suppressed Wnt/ β -catenin pathway *in vivo* and augmented the potency of chemotherapy

To further test the *in vivo* potency of lacs-miR-30C, we comparatively explored its anti-cancer action with chemotherapeutic agent, and further investigated its synergistic sensitized effect with chemotherapy. Towards this end, a patient-derived-tumor-xenograft (PDX) model of colon carcinoma was established through subcutaneously transplanting surgically acquired colon tumor into the fossa iliaca of NOD/SCID mice (Figure 5A). In this model, the acquired colon tumor was characterized by identifying missense mutated oncogenes, including PIK3CA (S66T), APC (V1822D), EGFR (R521K), KRAS (G12D) (Figure 5B). Of note, V1822D mutation of APC suggested the hyper-action of Wnt signaling pathway, and G12D mutation of KRAS means the high malignancy and clinical incurability. When the tumor volume reached $100 \pm 30 \text{ mm}^3$, tumor-bearing mice were treated with NS (Control), lacs-miR-30C, 5-FU (the first-line chemotherapy agent for colon cancer), or lacs-miR-30C/5-Fu combo every other day for 13 days, involving intravenous injections at the dosage of 2 mg/kg lacs-miR-30C, and/or of 5 mg/kg of 5-FU (Figure 5A). At day 5, immunohistochemical staining image of tumors revealed that lacs-miR-30C remarkably down-regulated Bcl9 and effectively suppressed the β -catenin/Wnt signaling cascade as support by the decreased protein level of Bcl9, β -catenin, and two Wnt- downstream proteins, C-myc and Cyclin D (Figure 5C). As a result, compared with mock-treated group, lacs-miR-30C statistically

significantly suppressed the tumor growth in more action than 5-Fu as evidence by the tumor volume curve (Figure 5D), tumor growth inhibition (TGI) effect (Figure 5E), tumor photos (Figure 5F) and tumor weights (Figure 5G). More importantly, the combination therapy between lacs-miR-30C and 5-Fu showed a significant increase anti-tumor action in contrast to the monotherapy of lacs-miR-30C or 5-Fu (Figures 5D-G), suggesting their synergistic sensitized effect. Moreover, proliferative cells analysis (ki67 staining) and apoptotic cells analysis (TUNEL staining) also supported these results (Figures 5H and I). Collectively, our data illustrated that lacs-miR-30C potentially suppressed Wnt/ β -catenin pathway *in vivo* and augmented the anti-tumor action of the chemotherapeutic agent 5-FU.

lacsRNA sensitized tumor immunotherapy

Programmed cell death ligand 1 (PD-L1) on tumor cells or tumor derived exosomes binds programmed cell death 1 (PD-1) on T cells and efficiently stimulates cytotoxic CD8⁺T cell malfunctioning and apoptosis, allowing cancer cells to escape from the immune attack [17, 35, 36]. Anti PD-1 antibodies or anti PD-L1 antibodies are thus considered as effective anti-tumor drugs, but its anti-tumor response is always restrained to T cell infiltration. In tumor, Wnt signaling results in T-cell exclusion and resistance to anti-PD-L1 or anti-PD-1 therapy [37]. Thus, we speculated that suppressing Wnt signaling cascade by lacs-miR-30C can sensitized the PD1/PD-L1 immuno-checkpoint blocking therapy. To verify it, we established an immunotherapeutic model in which C57/B6L mice were subcutaneously inoculated with B16F10 cells on the flank and treated with lacs-miR-30C, anti-PD-1 antibody or lacs-miR-30C/Anti-PD-1 antibody combo. As shown in Figures S4 and S5, lacs-miR-30C suppressed the β -catenin/Wnt signaling cascade in B16F10 tumor as support by the decreased protein level of Bcl9, β -catenin, C-myc and Cyclin D. Not surprisingly, compared with Anti-PD-1 or lacs-miR-30C monotherapy, the combo treatment resulted in a significantly decreased amounts of regulatory T lymphocyte (CD4⁺/CD25⁺ cells, Figure. 6A, B) in sharp contrast to an increased number of tumor-infiltrating cytotoxic T lymphocyte (CD3⁺/CD8⁺ cells, Figure. 6C, D). The inhibited tumor cell proliferation (Figure 6E) and the enhanced tumor cell apoptosis (Figure 6F) also supported the synergistic effect of lacs-miR-30C on anti-PD-1 therapy. As a result, lacs-miR-30C/Anti-PD-1 combo therapy led to dramatically increased TGI value (94.3% TGI) as compared to anti-PD-1 therapy (37.5% TGI) or lacs-miR-30C therapy (62.7% TGI) (Figures 6G and H), in line with the results of the tumor photos (Figure 6I) and weights (Figure 6J). Collectively, these results provided abundant evidences that lacs-miR-30C treatment allowed overcoming T cell exclusion and amplified anti-tumor effects in anti-PD-1 antibody therapy through suppressing Wnt signaling cascade.

Discussion

Wnt signaling cascades play a crucial part in embryonic development, tissue homeostasis and stem cells proliferation and differentiation for all animals.[38] The aberrant activation of this signaling, however, underlies multiple human cancers, including but not limited to intestinal cancer, lung cancer, liver cancer and lymphoma.[39] In this case, dysregulated Wnt signaling cascades accumulates the transcriptional activator β -catenin in the cytoplasm and the nucleus, resulting in the promotion of the genes expression for malignant cell proliferation and metastasis. In the process, as a component of the aberrantly

activated Wnt signaling pathway, β -catenin coactivators, including Bcl9, are always overexpressed in a variety of malignancies, and compose a stable complex with β -catenin to increase such malignant gene expression. [40] What's worse, dysregulated Wnt signaling cascades will reduce the infiltration of chemotherapeutics and T-lymphocytes (T-cell), resulting in the resistance of the chemotherapies and PD-1/PD-L1 checkpoint-blockade Immunotherapies. [41–43] To address it, some researches have verified that the suppression of tumor-intrinsic active β -catenin signaling not only can inhibit the tumor progression, but also sensitized the chemotherapy and Immunotherapy ([44–47]). Of note, the present work amply proved these points: lacs-miR-30C effectively sensitized both potency of 5-Fu in PDX model of colon cancer (Figure 5) and Anti-PD1 in B16F10 homograft model of melanoma (Figure 6).

Although there has existed some success for Wnt/ β -catenin suppression, many still remain to be done, in especial of tumor specificity. The activated Wnt pathway is a common element in regulating stem cell/progenitor cell renewal and maintenance in noncancerous tissues and organs[48, 49]. As a results, Wnt/ β -catenin inhibitor always cause on-targeted toxicity, therefore none of them has yet been approved for clinical application [16]. To address this issue, Bcl9 that is highly expressed in tumors but not in the cells of tumor origin, has received considerable attention.[50] It has been reported that the oncogenic role of Bcl9 can be rescued by siRNA/ShRNA-induced knockdown or the treatment with stapled Bcl9 peptide, both of which attenuated proliferation, metastasis, and resistance to therapy, highlighting the importance of Bcl9 for targeted oncotherapy [51, 52]. Thus, the development lacs-miR-30C possibly supplies the gap of safe and effective Wnt inhibitor for clinical transformation.

Conclusion

Herein, a general method through a mild and simple chemistry was established to convert therapeutic miRNA into a stable and bioavailable lacsRNA. Driven by aurophilicity, lacsRNA self-assembled into a spherical nanostructure with high colloid as well as anti-hydrolysis stability and low macrophage uptakes. As a consequence, lacsRNA presented the increased half-life period in circulation and accumulation at tumor sites in comparison to normal miRNA. More importantly, lacs-miR-30c showed no toxicity of viscera and sanguis system in the 5-time injection dosage of the treatment. Expectedly, lacs-miR-30c potently suppressed the Wnt signaling pathway in vitro and in vivo, and effectively sensitized both potency of 5-Fu in PDX model of colon cancer and Anti-PD1 in B16F10 homograft model of melanoma. In short, this work amply confirmed the design of lacsRNA as a general and viable strategy of nano-pharmaceutic to concert flimsy therapeutic miRNA into potential drugs. Considering from a broader perspective, the miRNA-initiated infinite coordination self-assembly strategy has distinct advantages in resurrecting nuclear acid therapeutics, probably bringing new inspiration to RNA-derived therapeutics of a great variety of human diseases including cancer.

Declarations

Ethics approval and consent to participate

All animal experiments in this work were performed in accordance with the guidelines of the Animal Ethics Committee of Xi'an Jiaotong University (approval number: 2020-276), Xi'an, China.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing financial interest.

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Contributions

WH, JY and WL supervised and conceptualized the study, LL and WH performed most of the experiments, WH, LL and WL wrote the manuscript, WY helped with project design and provided guidance on some experiments. All authors read and approved the final manuscript.

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Figures

Figure 1

Fabrication and therapeutic mechanism of lacsRNA. (A) Illustration of the synthesis and tumor response procedure of lacsRNA. (B) TEM images of lacsRNA. (C and D) Hydrodynamic diameter (C) and ZETA potential (D) of lacsRNA measured in PBS buffer at pH 7.4. (E) FT-IR spectra of lacsRNA and RNA. The band at 1200 cm⁻¹ which was attributed to the stretching vibration of -SH. (F and G) S 2p XPS spectra (F) and Au 4f XPS spectra (G) of lacsRNA.

Figure 2

Physicochemical and pharmaceutical properties of lacsRNA. (A and B) Anti-degradation stability test (A) and quantitative analysis (B) of lacsRNA and nude miRNA by gel electrophoresis. (C and D) Flow cytometry analysis (C) and quantification (D) of different cells uptake of 1 μ M Cy3-labelled miRNA and lacsRNA after 6h incubations. (E) Colloidal stability of lacsRNA suspending in PBS containing 20% FBS at pH7.4 and 6.5 measured by DLS. (F) Blood-circulation curves of lacsRNA and RNA in healthy C57/B6 mice quantified by the fluorescence signal from the labeled Cy3 fluorescein in miRNA. (G) organ distribution of lacsRNA in BALB/c mice after systemic injection. Serial sacrifices were carried out at 2h, 24h, 48h and 72h after injection. Several organs/tissues, including heart, liver, spleen, lung, kidney and tumor were isolated to determine gold concentrations by ICP-MS. He, heart, Sp, spleen, Li, liver, Lu, lung, Ki, kidney, Tu, tumor. (H) tumor-to-organ ratios for lacsRNA at 2h, 24h, 48h and 72h after injection. (I and J) Tissue distribution (I) and quantification (J) of lacsRNA. The fluorescence signal from the tumors and normal organ after lacsRNA intraperitoneal injection (200 μ L, 1mM Au) at 1h. The data were presented as mean $\pm$ s.d. *, p <0.05, **, p < 0.01, ***, p < 0.001.

Figure 3

The biosafety evaluation of lacsRNA in vivo. (A) Hepatotoxicity testing of the lacsRNA measured by aspartate transaminase (ALT), alanine aminotransferase (AST), and pathological section of liver (scale bar: 100 μ m). (B) Nephrotoxicity testing of the lacsRNA measured by blood urea nitrogen (BUN), creatinine (CRE) and pathological section of kidney (scale bar: 100 μ m). (C) Analysis of red blood cell (RBC), white blood cell (WBC), lymphocyte (LYMPH), Neutrophil (NEUT), Platelets (PLT) and Hemoglobin (HGB) in mice

blood with the indicated treatments. (D) Body weight of mice with the indicated treatments. (E) Toxicity testing of the lacsRNA measured by pathological section of heart, lung and spleen. The data were presented as mean $\pm$ s.d. *, $p < 0.05$.

Figure 4

lacsRNA targeting Bcl9 inhibits Wnt pathway. (A and B) B16F10 cells were treated with lacsRNA and miR-30C for 24 h at the dosage of 50nM, and qRT-PCR was performed to analyze the expressions of miR-30C

(A) and Bcl9 (B). (C) Hierarchical clustering of genes differentially expressed in B16F10 cells after exposure to 50nM lacsRNA for 24 h compared with miR-30C-treated cells (n = 3). (D) Hierarchical clustering of genes in Wnt/ β -catenin signaling pathway. (E-J) Gene set enrichment analysis (GSEA) showing the Wnt/ β -catenin pathway, β -catenin signaling pathway and cell cycle differentially expressed in response to lacsRNA. KEGG, Kyoto Encyclopedia of Genes and Genomes, PID, Pathway Interaction Database, Nes, normalized enrichment score. (K) Cell cycle was analyzed by flow cytometry. The data were presented as mean $\pm$ s.d. *, $p < 0.05$, **, $p < 0.01$.

Figure 5

lacsRNA in vivo inhibited patient-derived tumor xenograft colon carcinoma in NOD/SCID mice. (A) Schematic diagram of PDX model construction. (B) Detection of tumor mutations by exon sequencing. WT stands for wild type. (C) Representative tumor sections after the indicated treatment staining by immunohistochemistry (scale bar: 100 μ m). (D) Tumor growth curves in PDX mouse model mice with the indicated treatments (n = 5). (E) Tumor growth inhibition (TGI) with the indicated treatment at day 13. (F and G) Representative photographs (F) and weight of tumor (G) after the indicated treatments. (H and I) Ki67 (H) and TUNEL (I) staining in tumor sections from mice with the indicated treatments (scale bar: 100 μ m). The data were presented as mean $\pm$ s.d. Statistical analysis was performed using t test, *, p < 0.05, **, p < 0.01, ***, p < 0.001.

Figure 6

lacsRNA enhanced anti-cancer activity of PD1 in C57 mice bearing B16F10 tumor. (A and B) Immunofluorescence images of CD3+/CD8+ cells (A) and quantification (B) in tumor sections from mice with the indicated treatments (scale bar: 50 μ m). (C and D) Immunofluorescence images of CD4+/CD25+ cells (C) and quantification (D) in tumor sections from mice with the indicated treatments (scale bar: 50 μ m). (E and F) Ki67 (E) and TUNEL (F) staining in tumor sections from mice with the indicated treatments

(scale bar: 50 μm). (G) Growth curves of B16F10 tumors in C57 mice with the indicated treatments (n = 5). (H) Tumor growth inhibition (TGI) with the indicated treatment at day 9. (I and J) Representative photographs (I) and weight of tumor (J) after the indicated treatments. The data were presented as mean $\pm$ s.d. Statistical analysis was performed using t test, *, p < 0.05, **, p < 0.01, ***, p < 0.001.

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