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TITLE: Ultrastable Nontoxic RNA Nanoparticles for Targeting Triple-Negative Breast Cancer Stem Cells

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14. ABSTRACT

While the underlying biological mechanisms of TNBC has been unraveled to a large extent over the last decade, effective strategies to deliver therapeutics to cancer cells *in vivo* has remained a great challenge. We have constructed a new generation of drugs composed purely of RNA that is capable of targeting and treating cancers utilizing the phi29 pRNA three-way junction (3WJ) motif. Here, we have applied our innovative non-toxic and non-immunogenic RNA constructs with specific TNBC targeting capability to deliver high doses of therapeutic payloads to the cancer cells, while hoping to exhibit little or no collateral damage to healthy tissues. During this reporting period, we have successfully constructed RNA nanoparticles (NP) with ligands proven to bind and internalize into Human breast cancer stem cells as confirmed by flow cytometry and confocal microscopy. The targeted nanoparticles were able to significantly silence marker gene or oncogenic miRNA *in vitro* using Human breast cancer stem cells. Furthermore, utilizing 3D culture models to closer reflect *in vivo* conditions, RNA nanoparticles were delivered to TNBC cultures showing successful therapeutic effect by studying cell proliferation, migration, and invasion. We showed that RNA nanoparticles we constructed did not show side effects as demonstrated by immunogenicity assay. These studies establish a baseline of future *in vivo* studies as the final stage of this contract, in which we expect RNA nanoparticles to specifically treat TNBC tumor models in mice, with no side effects.

15. SUBJECT TERMS
Triple negative breast cancer; RNA nanotechnology; three-way junction; RNA aptamer; miRNA; cancer stem cell; EGFR aptamer; CD133 aptamer; tumor targeting

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1. INTRODUCTION:

Triple negative breast cancer, accounts for 15%-20% of all breast cancer cases, is among the most aggressive breast cancers and associated with poor prognosis and short survival. Currently, TNBC patients usually receive pre-operative neoadjuvant treatment involving the administration of chemotherapeutic drugs before surgery. However, the outcome for the majority who still have residual disease after treatment is relative poor. Due to the dismal diagnosis and low efficacy of traditional treatments for TNBC patients, new targeted therapeutic strategies are critically needed. RNAi has emerged in the past decade as a promising approach for cancer treatment. However, the poor physicochemical properties of the naked RNAi agent result in quick degradation and elimination in blood circulation, thus lowering the bioavailability significantly. Therefore, we have proposed to utilize RNA nanotechnology to solve these problems and delivered therapeutic anti-miRNA to TNBC specifically and efficiently. During our funding period, we have successfully designed and constructed RNA nanoparticles (NP) harboring targeting ligands (EGFR aptamer and CD133 aptamer) and anti-oncogenic miRNA. The multivalent RNA nanoparticles can target TNBC cells and Breast Cancer Stem Cells (BCSC), inhibit oncogenic miRNA function and induce cancer cell apoptosis. No or low toxicity and immunogenicity were detected. We have established orthotopic TNBC mouse models and indicated the multifunctional RNA nanoparticles could not only accumulate in TNBC tumor but also inhibit tumor growth efficiently. Our studies revealed the great potential of RNA nanoparticles as a delivery platform and also provide a foundation for clinical trial entry in the future.

2. KEYWORDS:

RNA nanotechnology; RNA nanoparticles; three-way junction; EGFR aptamer; CD133 aptamer; anti-miRNA; triple negative breast cancer; breast cancer stem cell

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Design RNA constructs harboring imaging and aptamers targeting breast cancer metastatic cells. (Months ~1-3) (Shu)

- Completed.

Aim 2: Design RNA constructs harboring therapeutic anti-miRNAs for suppressing oncogenic TNBC genes. (Months ~3-6) (Shu)

- Completed.

Aim 3: Assessment of multifunctional therapeutic RNA nanoparticles in 2D/3D cell cultures and TNBC orthotopic mouse models. (Months ~6-36) (Shu and Xu)

- Completed.

What was accomplished under these goals?

Major achievements:

1) We have successfully constructed pRNA-3WJ nanoparticles (NP) carrying targeting aptamer (EGFR aptamer, CD133 aptamer), therapeutic anti-miR21 and near infra-red fluorophores (Alexa647) as the imaging module.
2) We have carried out comprehensive characterization analyses of pRNA-3WJ/$EGFR_{apt}$/anti-miR21 and pRNA-3WJ/$CD133_{apt}$/anti-miR21 nanoparticles, including the assembly, size, zeta-potential, serum stability and Tm.
3) We have evaluated RNA nanoparticles' binding and internalization capability to TNBC cells and BCSC (target by CD133 aptamer), as well as gene regulation and therapeutic effects of the incorporated anti-miR21 in cell cultures.
4) We have evaluated the bio-distribution profiles and therapeutic effects of pRNA-3WJ/$EGFR_{apt}$/anti-miR21 and pRNA-3WJ/$CD133_{apt}$/anti-miR21 nanoparticles in the well-established TNBC mouse model. Downstream marker expressions were also studied at the gene and protein levels.

5) We have shown that constructed RNA nanoparticles were immunogenicity inert and did not elevate tested cytokines induction including TNF-α, IL6, and IFN-γ .

Specific objectives:

1) To construct RNA nanoparticles that can specifically target TNBC MDA-MB-231 cells and BCSC.
2) To confirm the RNA nanoparticles' authentic folding, homogeneity, and stability (serum stability and thermodynamic stability) under physiological conditions.
3) To demonstrate *in vitro* cell entry, gene regulation, and cell proliferation profiles treated by RNA nanoparticles.
4) To demonstrate the *in vivo* targeting and tumor suppression ability of the constructed RNA nanoparticles in TNBC models.
5) To study immunogenicity of RNA nanoparticles by detecting several pro-inflammatory cytokines induction *in vitro* and *in vivo* followed by treatment.

Significant results:

1) To study the marker expression on cell surfaces, antibodies were used for binding affinity study assayed by flow cytometry. We demonstrated TNBC MDA-MB-231 cells have a relative high level of EGFR and CD44 expression, mild level of CD133 expression and low EpCAM expression. (**Fig. 1A**) EGFR, CD44 and CD133 are more appropriate to serve as potential target on TNBC cells. (**Shu lab**)

2) We demonstrated that pRNA-3WJ nanoparticles incorporated with EGFR, CD44 and CD133 aptamers exhibit higher affinity than those with EpCAM aptamers to bind to MDA-MB-231 cells by flow cytometry, which is consistent with cell surface marker expression. (**Fig. 1B**). (**Shu lab**)

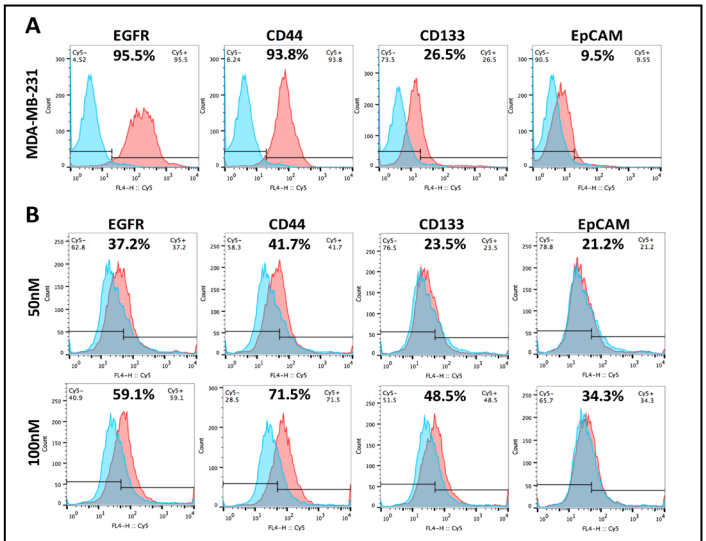

Fig. 1: Flow cytometry assay showing (A) expression of MDA-MB-231 cell surface marker expression, and **(B)** RNA NPs incorporated different aptamers ($EGFR_{apt}$, $CD44_{apt}$, $CD133_{apt}$ and $EpCAM_{apt}$) binding to MDA-MB-231 cells at 50nM and 100nM.

3) We have designed and constructed RNA nanoparticles carrying EGFR aptamers to target TNBC cells. The branched structure of pRNA-3WJ nanoparticles provide multivalence to incoporate functional modules. The multifunctional nanoparticles contained anti-miR21 as a therapeutic molecule, EGFR aptamer as a targeting ligand and Alexa647 fluorephore as an imaging agent. (**Fig. 2A**) Native PAGE has demonstrated the step-wise assembly of RNA nanoparticles. (**Fig. 2B**) The size of $3WJ/EGFR_{apt}$/anti-miR21 is about 14.8 ± 2.6nm and its zeta potential is -17 ± 5.6mV. (**Fig. 2C&D**) It was found that the Tm of $3WJ/EGFR_{apt}$/anti-miR21

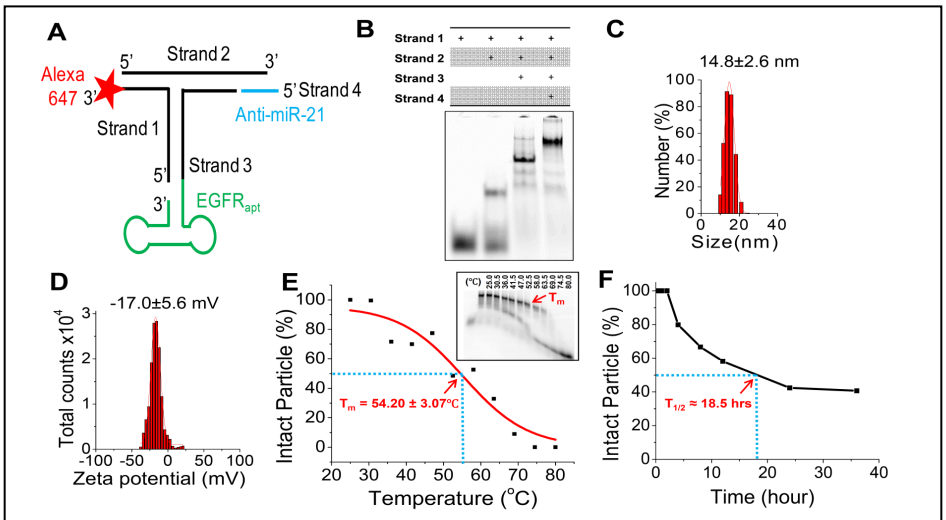

Fig. 2: Design and physicochemical characterization of $3WJ/EGFR_{apt}$/anti-miR-21 nanoparticles. (A) 2D sequence of the nanoparticle harboring three functional modules: EGFR RNA aptamer for targeted delivery, anti-miR-21 LNA for therapy, and Alexa-647 dye for imaging. **(B)** Native PAGE showing stepwise highly efficient assembly of the RNA nanoparticle. **(C)** DLS measurements showing the hydrodynamic size. **(D)** Zeta potential. **(E)** Serum stability. **(F)** Apparent Tm extracted from temperature gradient gel electrophoresis (TGGE, inserted).

nanoparticles was about 54°C (**Fig. 2E**), which is much higher than the physiological temperature. These RNA nanoparticles are stable in serum enviornment for a long time. (**Fig. 2F**)

4) The CD133 aptamer was also utilized as a targeting ligand built in pRNA-3WJ nanoparticles to target both TNBC cells and breast cancer stem cells (BCSC). (**Fig. 3A**) 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles revealed high efficient stepwise assembly in gel electrophoresis (**Fig. 3B**). DLS determined that the average hydrodynamic diameter of 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles was about 10.71 ± 2.846nm (**Fig. 3C**). The zeta potential was determined to be -25.3 ± 10.8mV (**Fig.3D**). They also exhibited high chemical stability and thermodynamic stability consistent our previous results. (Data not shown here)

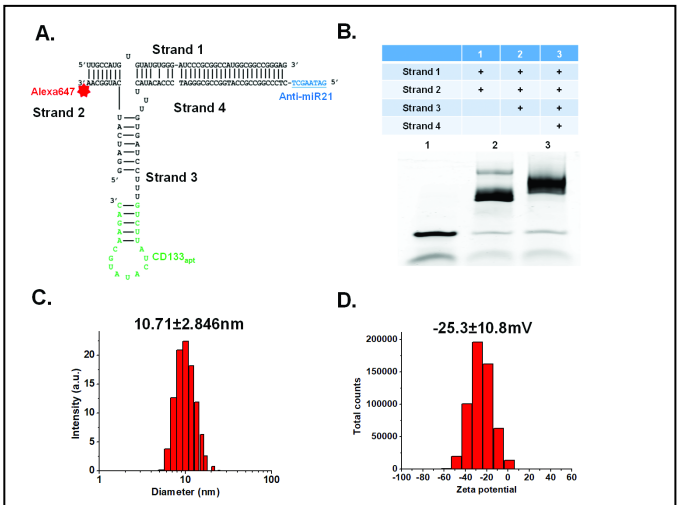

Fig. 3: Design, construction and characterization of RNA nanoparticles carrying anti-miR21 and CD133 aptamer. (A) 2D structure of 3WJ/CD133$_{apt}$/anti-miR21 (Blue underline sequence is 8 nt LNA modified anti-miR21; Green sequence is 19 nt CD133 aptamer). **(B)** Stepwise-assembly of 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles assayed by 12% TBE Native PAGE. **(C)** Size distribution of 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles. **(D)** Zeta-potential of 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles.

5) Both confocal microscopy and flow cytometry were used to study the binding affinity of RNA nanoparticles carrying aptamers. Our results demonstrated the RNA nanoparticles with either the EGFR aptamer or CD133 aptamer exhibited high affinity to TNBC MDA-MB-231 cells. Aptamer decorated RNA nanoparticles showed strong co-localization in the cell cytosol compared with control groups. (**Fig. 4A & 4C**) In addition, 3WJ/CD133$_{apt}$ nanoparticles can bind to BCSC and are distributed in cytosol. CD133 aptamer facilitates more nanoparticles to be internalized to both cell lines. Flow cytometry revealed consistent result that both 3WJ/EGFR$_{apt}$ and 3WJ/CD133$_{apt}$ nanoparticles had stronger binding ability to TNBC MDA-MB-231 cells compared with non-targeted 3WJ nanoparticles. (**Fig. 4B&4D**)

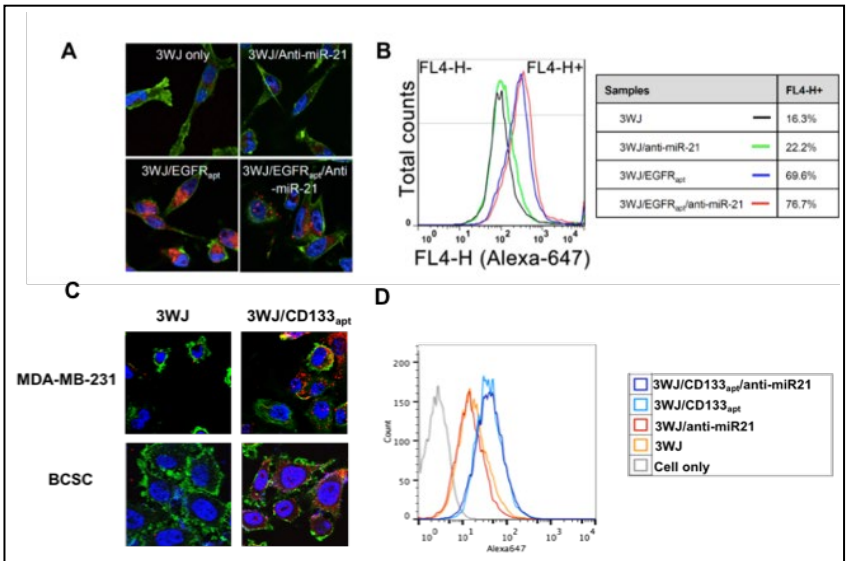

Fig. 4: Binding and internalization of RNA nanoparticles mediated by aptamer. (A) Internalization of RNA nanoparticles with EGFR$_{apt}$ to TNBC MDA-MB-231 cells. **(B)** Cell binding assay of RNA nanoparticles carrying EGFR$_{apt}$ by flow cytometry. **(C)** RNA nanoparticles' internalization profile in TNBC MDA-MB-231 and BCSC cells by confocal microscopy (Blue: nuclei, Green: cytoplasm, Red: RNA nanoparticles). (D) Cell binding assay of RNA nanoparticles carrying CD133$_{apt}$ by flow cytometry.

6) Our results (**Fig. 5A**) demonstrated delivery of anti-miR21 by CD133 aptamer showed higher R/F luc ratio on both TNBC MDA-MB-231 cells and BCSC in a dose dependent manner, indicating that the CD133 aptamer mediated the favorable delivery of anti-miR21 and subsequently inhibited the activity of endogenously oncogenic miR21. In our study, we found obvious upregulation of PTEN and PDCD4 after treatment by 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles assayed by q-PCR (**Fig. 5B**). Upregulation of PTEN and PDCD4 can induce cell apoptosis and inhibit cell proliferation. Treatment by 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles reduced the invasive property of MDA-MB-231 cells from the analysis of the representative images taken by Inverted Microscopy (**Fig. 5C**). Less crystal staining was found compared to the control groups.

7) We demonstrated the successful construction of RNA nanoparticles (NP) that have therapeutic effects on TNBC, as revealed by the 3D cultures model (**Fig. 6**). A 3D culture is a physiologically relevant tissue culture model to investigate cancer progression. MDA-MB 231 cells were treated with 3WJ RNA nanoparticles for 2 days, and then were cultured in Matrigel for three days. The invasive branching structures and cell migration were assessed. Our results showed that EGFR, CD133, and CD44 aptamer delivered RNA nanoparticles repressed invasive growth of TNBC cells in 3D culture (**Fig. 6**)

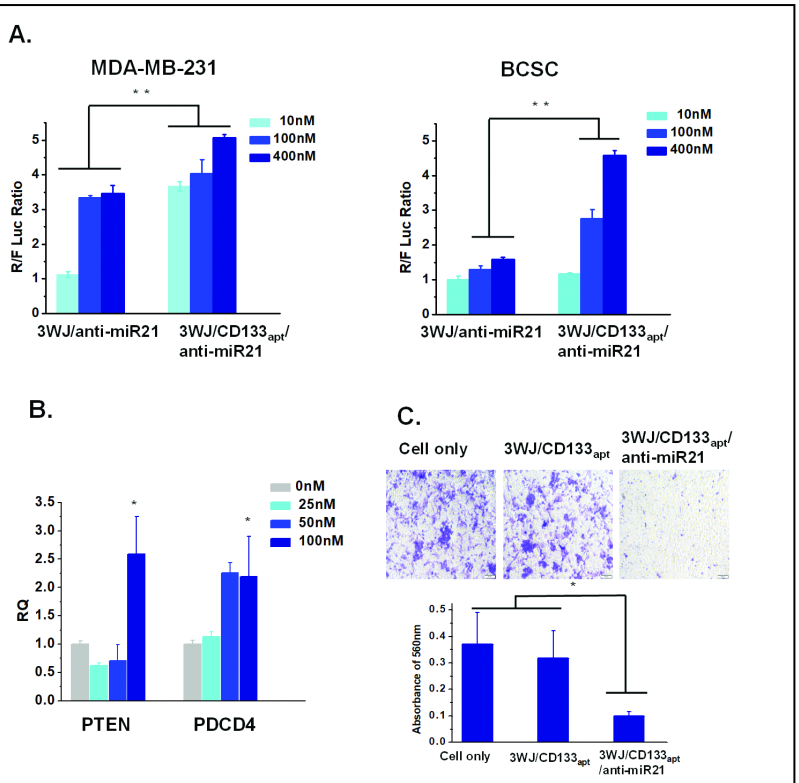

Fig. 5 Therapeutic effects of 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles *in vitro*. **(A)** Dual-luciferase assay showing delivery of anti-miR21 to TNBC MDA-MB-231 and BCSC cells. (**B)** qRT-PCR demonstrating downstream gene regulation. (**C)** Cell migration study (Upper panel: representative images taken by inverted microscopy, down panel: crystal violet quantification by plate reader). In all plots, data are represented as mean ± SD. *p <0.05, **p<0.01.

8) We demonstrated that the 3WJ/EGFR$_{apt}$/anti-miR21 RNA nanoparticle can suppress tumor growth in orthotopic TNBC mouse model. The end point luminescence signal, which reflects the tumor volume, from the mice treated with this RNA nanoparticle was significantly lower than the one from mice treated with control RNA nanoparticles (**Fig. 7A**). The tumor growth curve also showed the sustained inhibition of tumor growth by 3WJ/EGFR$_{apt}$/anti-miR21, RNA nanoparticles compared to the control (**Fig. 7B**). We demonstrated that the 3WJ/EGFR$_{apt}$/anti-miR21 RNA nanoparticle can successfully target TNBC tumor in orthotopic TNBC mouse models after systemic injection, with little accumulation in other healthy organs 8 hours post injection. Strong targeting of the RNA nanoparticles to the tumor cells was confirmed by *ex vivo* fluorescence images of organs (**Fig. 7C**) and tissue section (**Fig. 7D**). We further validated the anti-miR-21 knockdown at the molecular level after the systemic injection of the 3WJ/EGFR$_{apt}$/anti-miR21 RNA nanoparticle in TNBC mouse model. The miR-21 as well as its downstream target mRNAs of PTEN and PDCD4 were quantified by qRT-PCR assay at the mRNA level, and the expression of PTEN and PDCD4 was also examined by western blot assay at the protein level. The miRNA expression was reduced, while PTEN and PDCD4 expression was enhanced in 3WJ/EGFR$_{apt}$/anti-miR21 treated tumors (**Fig. 7E-F**). After treatment, the tumor cells showed lower expression level of Ki-67, a cellular marker for cell proliferation (**Fig. 7G**), and increased expression of Caspase-3, an apoptosis indicator (**Fig. 7G**). (**Collaboration between Shu and Xu labs**)

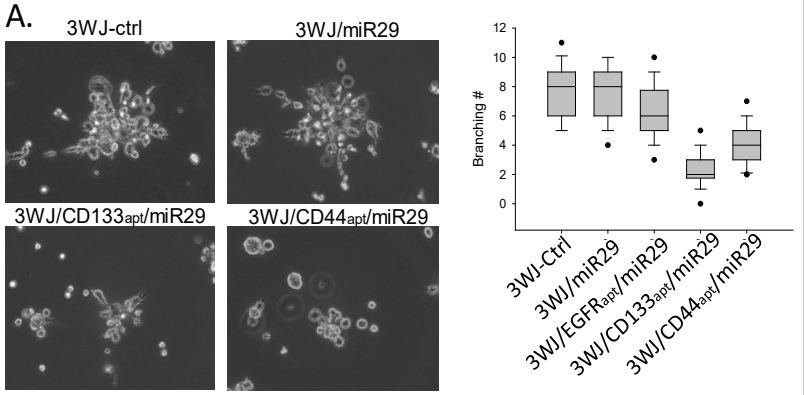

Fig. 6: Tumorsphere assay to examine the inhibitory activity of RNA nanoparticles on self-renewal tumor initiating capacity of TNBC. 3D cell cultures were created, allowing TNBC cells to organize into invasive structures to resemble *in vivo* phenotypes. After MDA-MB 231 cell were cultured in the presence of RNA nanoparticles for 48h, the cells were harvested and cultured in 3D Matrigel in the presence of NP. Images were taken after 3 days in 3D culture (A), and invasive branching structures (B) were quantified.

9) Animal studies have also been used for RNA nanoparticles with CD133 aptamer. The bio-distribution study demonstrated that 3WJ/CD133$_{apt}$ nanoparticles exhibited strong fluorescence signal in tumors with little or no signal in other healthy organs at 8 hours post injection (**Fig. 8A**). The therapeutic effect of 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles was validated by a tumor inhibition study in orthotropic TNBC model in nude mice. Treatment with nanoparticles significantly inhibited tumor growth in the MDA-MB-231 xenograft model compared with the control group (**Fig. 8B**). The data revealed that the 3WJ/CD133$_{apt}$/anti-miR21 nanoparticle treatment upregulated PTEN and PDCD4 expression in tumor tissues compared to PBS control. (**Fig. 8C**) Furthermore, the treatment by RNA nanoparticles enhanced cell apoptosis, as indicated by increased active caspase3 (Act-caspase3) levels in histological analysis (**Fig. 8D**).

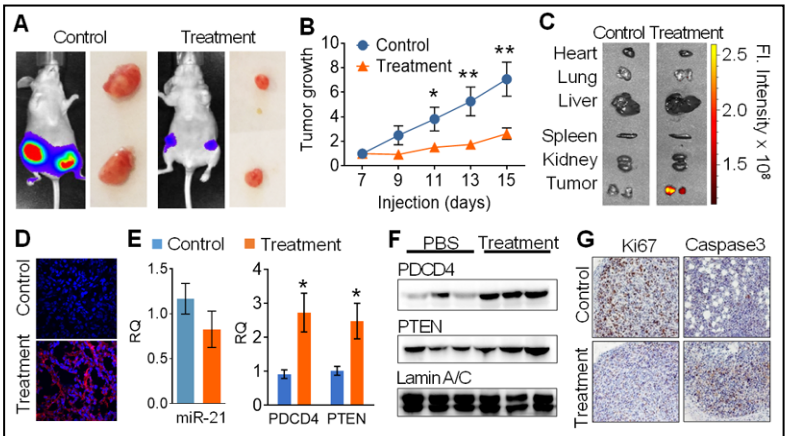

Fig. 7: Evaluation of targeting and therapeutic effects of pRNA-3WJ nanoparticles *in vivo* in TNBC mouse xenograft. (A) Tumor inhibition over the treatment course. The endpoint luciferase luminescence indicates the tumor volume. **(B)** Plot of tumor growth over treatment course. (*P<0.05, **P<0.01). **(C)** Fluorescent images showing the specific targeting of the RNA nanoparticle (Red) to TNBC tumor. **(D)** Binding and internalization of the RNA nanoparticles (Red) to tumor tissue. Blue: Nuclei. **(E)** qRT-PCR results showing down-regulation of miR21 and up-regulation of two targeting genes PTEN and PDCD4 after treatment. **(F)** Western blot showing the up-regulation of PTEN and PDCD4. **(G)** Immunohistochemistry data of two markers indicating the growth inhibition of tumor cells after treatment.

10) We tested the immunogenicity of RNA nanoparticles by cytokine induction assays. The results (**Fig. 9A**) showed that 3WJ/CD133$_{apt}$/anti-miR21 at therapeutic concentration (1 μM) induced neither TNF-α nor IL6 production compared to LPS positive control while incubating with mouse macrophage-like RAW 264.7 cells in vitro. Furthermore, all three cytokines were undetectable compared to LPS positive control after systemic injection into CD-1 mice (**Fig. 9B**). The low induction of cytokine suggests that RNA as biomaterials is biocompatible and relatively safe.

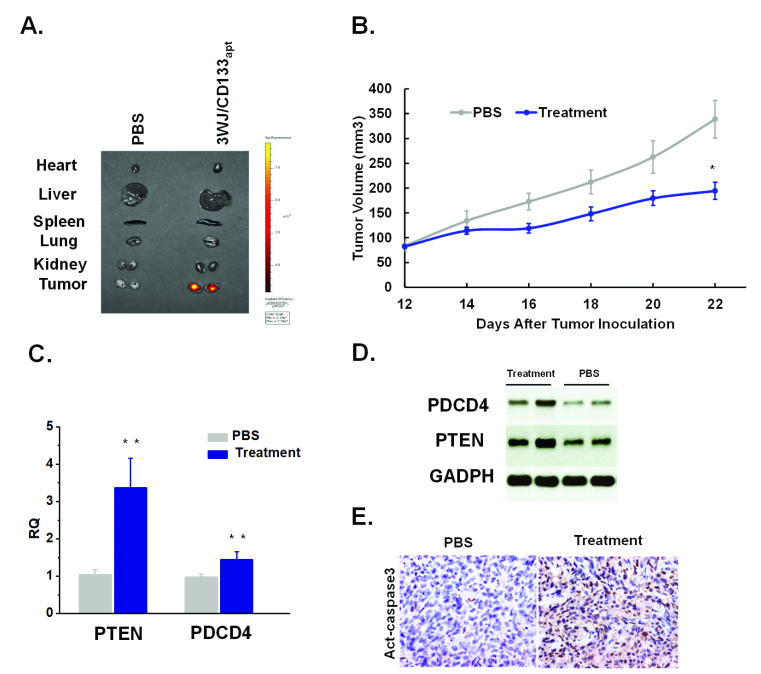

Fig. 8: Evaluation of targeting and therapeutic effects of 3WJ/CD133$_{apt}$/anti-miR21 nanoparticles in animal trial. A. Fluorescence image showing RNA nanoparticles distribution in organs at 8 hr post IV injection. **B.** TNBC tumor growth curve over the course of 5 injections. (Data are represented as mean ± SEM *p<0.05) **C.** qRT-PCR assay to study downstream gene expression level in mRNA level. (Data are represented as mean ± SD. **p<0.01) **D.** Western blot assay to study downstream gene expression in protein level. **E.** Immunohistochemistry assay demonstrating Act-caspase3 level in tumor.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

1) We have published the results in scientific journals:

Chen J, Wang S, Zhang Z, and Richards CI, Xu R. Heat shock protein 47 (HSP47) binds to discoidin domain-containing receptor 2 (DDR2) and regulates its protein stability. J Biol Chem. 2019 Sep 30. PMID: 31570520

Zheng Z, Li Z, Xu C, Guo B, Guo P. Folate-displaying Exosomes Mediated Cytosolic Delivery of siRNA Avoiding Endosome Trapping. Journal of Controlled Release. J Control Release. 2019 Aug 22;311-312:43-49.

Xu Y, Pang L, Wang H, Xu C, Shah H, Guo P, Shu D, Qian ST. Specific Delivery of Delta-5-desaturase siRNA via RNA Nanoparticles Supplemented with Dihomo-γ-linolenic Acid for Colon Cancer Suppression. Redox Biology. 2019; 21:101085.

Yin H, Xiong G, Guo S, Xu C, Xu R, Guo P, Shu D. Delivery of Anti-miRNA for Triple Negative Breast Cancer Therapy Using RNA Nanoparticles Targeting to Stem Cell Marker CD133. Molecular Therapy. 2019 July; 27(7):1252-1261.

Zhang H, Fredericks T, Xiong G, Qi Y, Li J, Pihlajaniemi T, Xu W, and Xu R, Membrane associated collagen XIII promotes cancer metastasis and enhances anoikis resistance. Breast Cancer Research. 2018; 20: 116

Yin H, Wang H, Li Z, Shu D, Guo P. RNA micelles for systemic delivery of anti-miRNA for cancer targeting and inhibition without ligand. ACS Nano. 2019 Jan 22;13(1):706-717.

Wang S, Li Z, Xu R. Human Cancer and Platelet Interaction, a Potential Therapeutic Target. International Journal of Molecular Sciences. 2018; 19(4):1246.

Qi Y, Xu R. Roles of PLODs in Collagen Synthesis and Cancer Progression. Frontiers in Cell and Developmental Biology. 2018 June; 6:66.

Xiong G, Stewart RL, Chen J, Gao T, Scott TL, Samayoa LM, O'Connor K, Lane AN, Xu R. Collagen prolyl 4-hydroxylase 1 is Essential for HIF-1α Stabilization and TNBC Chemoresistance. Nature Communications. 2018; 9:4456.

Shu Y, Yin H, Rajabi M, Li H, Vieweger M, Guo S, Shu D, Guo P. RNA-based micelles: A novel platform for paclitaxel loading and delivery. J Control Release. 2018 Feb 14; 276:17-29.

Xu C, Haque F, Jasinski DL, Binzel DW, Shu D, Guo P. Favorable biodistribution, specific targeting and conditional endosomal escape of RNA nanoparticles in cancer therapy. Cancer Letters. 2017 Oct 5; 414:57-70.

Xu R. Mammary Epithelia Polarity and Macrohpahe Infiltration. Macrophage (Houst). 2017; 4:1521.

Li L, Chen J, Xiong G, St Clair DK, Xu W, Xu R. Increased ROS production in non-polarized mammary epithelial cells induces monocyte infiltration in 3D culture. J Cell Sci. 2017 Jan 1;130 (1):190-202.

Yang X, Xu Y, Wang T, Shu D, Guo P, Miskimins K, Qian SY. Inhibition of cancer migration and invasion by knocking down delta-5-desaturase in COX-2 overexpressed cancer cells. Redox Biol. 2017 Jan 26; 11:653-662.

Lee TJ, Yoo JY, Shu D, Li H, Zhang J, Yu JG, Jaime-Ramirez AC, Acunzo M, Romano G, Cui R, Sun HL, Luo Z, Old M, Kaur B, Guo P, Croce CM. RNA Nanoparticle-Based Targeted Therapy for Glioblastoma through Inhibition of Oncogenic miR-21. Mol Ther. 2017 Jul 5;25(7):1544-1555.

Zhang Y, Leonard M, Shu Y, Yang Y, Shu D, Guo P, Zhang X. Overcoming Tamoxifen Resistance of Human Breast Cancer by Targeted Gene Silencing using Multifunctional pRNA Nanoparticles. ACS Nano. 2017 Jan 24;11(1):335-346.

Li H, Zhang K, Pi F, Guo S, Shlyakhtenko L, Chiu W, Shu D, Guo P. Controllable Self-Assembly of RNA Tetrahedrons with Precise Shape and Size for Cancer Targeting. Adv Mater. 2016 Sep;28(34):7501-7.

Xiong GF, Xu R. Function of Cancer Cell-derived Extracellular Matrix in Tumor Progression. Journal of Cancer Metastasis and Treatment. 2016; 2:357-364.

Xiong G, Flynn TJ, Chen J, Trinkle C, Xu R. Development of an ex vivo breast cancer lung colonization model utilizing a decellularized lung matrix. Integr Biol (Camb). 2015 Dec; 7(12):1518-25.

Shu D, Li H, Shu Y, Xiong G, Carson WE, Haque F, Xu R, Guo P. Systemic Delivery of Anti-miRNA for Suppression of Triple Negative Breast Cancer Utilizing RNA Nanotechnology. ACS Nano. 2015 Oct 27; 9(10):9731-40

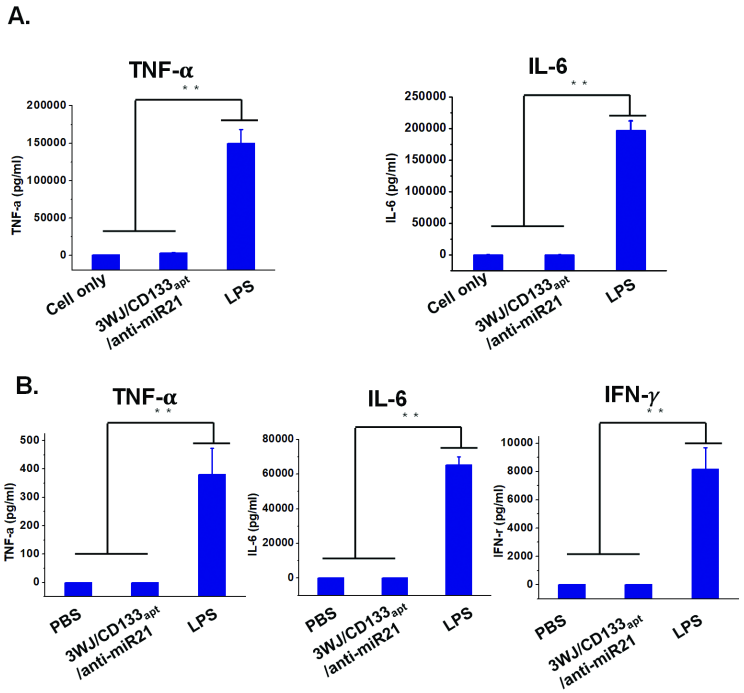

Fig. 9: Immunogenicity study of RNA nanoparticles *in vitro* and *in vivo*. (A) TNF-α and IL-6 induction in RAW264.7 cells after treatment. **(B)** TNF-α, IL-6 and IFN-γ level in CD-2 mice serum after treatment. In all plots, data are represented as mean ± SD. **p<0.01.

We have presented the results at scientific conferences:

Shu D, Li H, Shu Y, Xiong G, Carson WE, Haque F, Xu R, Guo P. Systemic Delivery of Anti-miRNA for Suppression of Triple Negative Breast Cancer Utilizing RNA Nanotechnology. IEEE-NANOMED 2015.

Shu D, Binzel D, Lee T, Yin H, Shu Y, Li H, Xu R, Guo B, Croce C, Guo P. RNA Nanotechnology for the Specific Delivery of Anti-miRNA for Suppression of Breast, Prostate and Brain Cancer. Gordon Research Conference. 2017. Ventura, CA.

Shu D, Binzel D, Lee T, Yin H, Shu Y, Li H, Xu R, Guo B, Croce C, Guo P. RNA Nanotechnology for the Specific Delivery of Anti-miRNA for Suppression of Breast, Prostate and Brain Cancer. The NanoWorld Conference. 2018. San Francisco, CA.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Safe and efficient delivery system is urgently needed to treat TNBC. RNAi based therapeutics have a great potential in cancer treatment. However, several challenges including rapid degradation *in vivo*, quick elimination from kidneys, non-specific binding, and gene regulation still hinder its clinical translation. Our study here demonstrated that pRNA-3WJ nanoparticles can be used as an effective delivery system for RNAi therapeutics. The stability of the 2'F modified pRNA-3WJ motif was improved significantly while retaining authentic function. In addition, it increases the size of the small RNAs and introduces cancer specific targeting moieties for enhanced pharmacokinetic and therapeutic efficacies. The results demonstrate the clinical potentials of the RNA nanotechnology based platform to deliver miRNA-based therapeutics for cancer treatment.

What was the impact on other disciplines?

The pRNA-3WJ nanoparticles are easy to make based on RNA nanotechnology. Multivalent property allows for the incorporation of a variety of functional modules including therapeutics, targeting ligands and imaging modules. The system can be applied for the treatment of different other diseases.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There have been no changes in approach.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

No cost extension was filed and approved extending the term of the project to 8/31/2019. However, there is no change in the expenditures as a result of the NCE filing.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Publications:

Chen J, Wang S, Zhang Z, and Richards CI, Xu R. Heat shock protein 47 (HSP47) binds to discoidin domain-containing receptor 2 (DDR2) and regulates its protein stability. J Biol Chem. 2019 Sep 30. PMID: 31570520

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- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

MiRNA for treatment of breast cancer. U.S. Patent Application PCT/US16/21451. 03/09/2016.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	**Dan Shu**
Project Role:	PI
Researcher Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	18
Contribution to Project:	Dr. Shu led and designed the studies in construction of RNA nanoparticles, and evaluation of the constructs in cell targeting and gene knockdowns.
Funding Support:	n/a

Name:	**Hui Li**
Project Role:	Graduate Student
Researcher Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	5.5
Contribution to Project:	Hui Li assisted Dr. Shu in the evaluation of the RNA constructs in gene knockdowns.
Funding Support:	n/a

Name:	**Zhefeng Li**
Project Role:	Graduate Student
Researcher Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	3
Contribution to Project:	Zhefeng Li assisted Dr. Shu in construction of the designed RNA constructs and *in vitro* evaluations of the constructs in cell cultures.
Funding Support:	n/a

Name:	**Hongran Yin**
Project Role:	Graduate Student
Researcher Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	23
Contribution to Project:	Hongran Yin assisted Dr. Shu in the evaluation of the RNA constructs in gene knockdowns.
Funding Support:	n/a

Name:	**Pu Zhang**
Project Role:	Graduate Student

Researcher Identifier (e.g., ORCID ID): n/a
Nearest person month worked: 5
Contribution to Project: Pu Zhang assisted Dr. Shu in construction of the designed RNA constructs and *in vitro* evaluations of the constructs in cell cultures.
Funding Support: n/a

Name: **Daniel Binzel**
Project Role: Postdoctoral Researcher
Researcher Identifier (e.g., ORCID ID): n/a
Nearest person month worked: 0.56
Contribution to Project: Daniel Binzel assisted Dr. Shu in the evaluation of the RNA constructs in gene knockdowns.
Funding Support: n/a

Name: **Ren Xu**
Project Role: Partner PI
Researcher Identifier (e.g., ORCID ID): n/a
Nearest person month worked: 2
Contribution to Project: Dr. Xu designed the animal studies to test *in vivo* function of RNA nanoparticles.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Dr. Dan Shu has received other funding supports during the reporting period:

P50CA168505 PI: Ringel 08/01/2016 – 07/31/2018 1.2 calendar
NIH/NCI $100,000 to Shu lab
Title: RNA Nanotechnology-based Therapy for Thyroid Cancer
Goal: The main goal is to develop RNA nanotechnology-based therapy for thyroid cancer.
Role: Project PI

R21CA209045 PI: Xu; Shu 09/01/2016 – 08/31/2018 1.2 calendar
NIH/NCI $39,673 to Shu lab
Title: Systemic delivery of miR-29 for basal-like breast cancer treatment
Goal: The main goal is to develop RNA nanoparticles harboring therapeutic miR-29b that will target basal-like breast cancer cells and evaluate the biologic activity of multifunctional therapeutic RNA nanoparticles in the orthotropic mouse mammary tumor model.
Role: MPI

1R01CA195573-01 PI: Evers, BM; Guo, P; Thorson, J 09/25/2015 – 08/31/2020 1.2 calendar
NIH/NCI $276,813 (total annual direct)
Title: Novel pRNA nanoparticle delivery as directed therapy for colorectal cancer metastasis
Goal: The main goal is to develop a highly effective and less toxic approach to specifically deliver chemotherapeutic agents to selectively target and inhibit colorectal cancer metastasis.
Role: Co-Investigator

1R01CA186100-01 PI: Guo, Bin 04/01/2015 – 03/31/2019 3.6 calendar
NIH/NCI $283,606 (total annual direct)
Title: RNA therapeutics for targeted treatment of colon cancer.
Goal: The main goal is to develop a new therapeutic strategy to deliver miR-627 and JMJD1A-targeting siRNA to inhibit colon cancer growth.
Role: Co-Investigator

What other organizations were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS:
Nothing to Report.

9. APPENDICES:
A copy of the recent publications is attached.