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Targeted Chimera Delivery to Ovarian Cancer Cells by Multifunctional Ruthenium Polypyridine Nanoparticles

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Abstract:

Background: In vivo efficient delivery of small interfering RNAs (siRNAs) to the targeted tumor cells has remained a significant challenge in clinical applications. Ruthenium (II) polypyridyl complexes have emerged both as anticancer agents and imaging theranostic agents because of unique photophysical and cytotoxic properties. However, a major limitation in their clinical implementation is effective cancer cell selectivity. In the present study, we present a novel targeted siRNA delivery platform obtained by ruthenium polypyridine complex nanoparticles (RPN).

Results: The chimera molecules constructed by EGFR RNA aptamer and Notch3 siRNA, the chimera loaded RPN exhibited much higher silencing efficiency against Notch3 gene compared with chimera alone and lipofectamine-siRNA complex, and improved the antitumor effects of chimera in vitro and in vivo against ovarian cancer, indicating its potential capability for future targeted cancer therapy via RPN mediated chimera delivery.

Conclusions: Overall, this work describes, to the best of our knowledge, the first in vivo study demonstrating the targeted chimera delivery into tumor cells by multifunctional ruthenium polypyridine nanoparticles.

Key words：Ruthenium Polypyridine Complex Nanoparticles (RPN); Chimera; Notch3; EGFR; Ovarian cancer

Background

Ovarian cancer is one of the most common malignant tumor endangering women's health, it has the highest mortality in the female reproductive system. At present, active tumor cell reduction and postoperative platinum based combined chemotherapy are the standard treatment for ovarian cancer[1-2]. However, chemoresistance has increasingly become a huge clinical problem. Some patients show chemoresistance during chemotherapy, resulting in treatment failure, or relapse after chemotherapy[3].

RNA interference (RNAi) is a post-transcriptional mechanism that involves the inhibition of gene expression through promoting cleavage on a specific area of a target messenger RNA (mRNA). RNAi has shown promising therapeutic results for a good number of diseases, especially in cancer. siRNA drugs derived from RNA interference effect can specifically silence genes related to cancer occurrence, development and metastasis, inhibit tumor cell proliferation, differentiation and metastasis, play a therapeutic role, and bring new hope for tumor treatment[4]. Due to the poor stability of siRNA, it is easy to be degraded by nuclease in serum and by lysosomal enzymes in lysosomes, which poses a great challenge to the clinical application of siRNA[5]. Therefore, the development of an effective siRNA delivery system that can protect siRNA targeting into cells is very important for the success of siRNA therapy[6].

The application of nanomaterials in siRNA transfection has attracted extensive attention in recent years, such as lipid and polymer nanoparticles[7], gold nanoparticles (AuNPs), magnetic nanoparticles and mesoporous silicon nanoparticles[8-10], which benefits from its high loading rate, good biocompatibility and biodegradability. To achieve the clinical potential of RNAi, delivery nanomaterials are required to transport short interfering RNA (siRNA) to the site of action in the cells of target tissues[11].

Ruthenium (II) polypyridine complexes are one of the most extensively studied and developed systems in the family of luminescent transition-metal complexes, and broadly applied as excellent antitumor agents[12], theranostic agents[13] and photodynamic therapy[14]. However, no study was reported for RNAi therapy mediated by ruthenium (II)

polypyridine up to now. Ruthenium (II) polypyridine based nanomaterials could be promise theranostic agents combined its antitumor activity and luminescent properties.

Nucleic acid aptamer is an oligonucleotide sequence that can specifically bind to target molecules as traditional antibodies, but offer several advantages, including their relatively small physical size, flexible structure, quick chemical production, versatile chemical modification, high stability and lack of immunogenicity[15]. In addition, many aptamers are internalized upon binding to cellular receptors, making them useful targeted delivery agents for small interfering RNAs (siRNAs) [16-18].

EGFR aptamer had been used as ligand for targeted drug delivery to cancer cells by nanoparticles in our previous work[19]. In this study, we used EGFR RNA aptamer and Notch3 siRNA to construct nucleic acid chimera molecule. Chimera can not only target EGFR (epithelial growth factor receptor 1) highly expressed in ovarian cancer cell membrane, but also silence Notch3, inhibit cell proliferation and induce apoptosis. As a siRNA delivery system, we synthesized a kind of multifunctional ruthenium polypyridine nanoparticles (RPN). The fluorescent nanoparticles have high chimera binding efficiency, the chimera carried on the nanoparticles has been well protected and the stability has been greatly improved. Chimera loaded RPN can be used for efficient targeted therapy of ovarian cancer in vitro and in vivo.

Results and Discussion

As shown in Figure 1A, RPN are dissolved in DMSO and the solution is orange red. TEM image (Figure 1B) shows that the particle size is relatively uniform. The hydraulic particle size is mainly concentrated among 50-110 nm (Figure 1C). The particle size is small, the specific surface area is large, and it is conducive to improve the absorption and bioavailability of drugs. The results of UV-Vis spectra show that the absorption peak at 264 nm is the characteristic absorption peak of intra-ligand charge transfer (IL), and the characteristic absorption peak of metal-ligand charge transfer (MLCT) is at 469.5 nm, which is the absorption peak of ruthenium (Figure 1D).

Moreover, the nanoparticles have their own fluorescence, ExMax is about 460-470nm, EmMax is about 595-605nm, and the fluorescence excitation state is red. It is conducive to drug tracing and evaluation of therapeutic effect.

The mass spectra (Figure S1A) in acetonitrile exhibited two peaks at m/z 809.3 (100%) and m/z 405.3 (33%) for RPN which was ascribed to $[M\text{-}2ClO_4\text{-}H]^+$ and $[M\text{-}2ClO_4]^{2+}$ and agreed with the theoretical value. The CD spectrum (Figure S1B) shows positive values at 298nm. The chemical shift(δ) of RPN in 1H NMR at 8.90 (d, J = 8.2 Hz, 2H) , 8.86 (d, J = 8.2 Hz, 2H) , 8.41 (d, J = 8.3 Hz, 2H), 8.25 (m, 2H) , 7.47 (dd, J = 4.8, 1.7 Hz, 2H), 7.37 (t, J = 6.5 Hz, 2H), 7.98 (d, J = 4.4 Hz, 2H) ppm can be attributed to fourteen H atoms in bpy ligands, respectively. The chemical shift at 9.07 (d, J = 8.1 Hz, 2H), 8.12 (t, J = 7.4 Hz, 2H) ppm can be attributed to the structure of phenanthroimidazole. The chemical shift at 7.88 (t, 6H) ppm can be attributed to the 3-position of bipyridine and the b-position of phenanthroimidazole and 7.78 (d, J = 7.9 Hz, 2H), 7.6-7.53 (dd, 6H) ppm can be attributed to hydrogen on benzene ring.

The chemical shift(δ) of L0627 in 13C NMR at 157.20 (s) ppm can be attributed to C1 and C10 (Figure S1C). The chemical shift at 151.79 (s) ppm can be attributed to C5、C6 and C14. The chemical shift at 144.74 (s),138.21 (s), 132.28 (s), 131.84 (s), 130.60 (s), 129.30 (s) ppm can be attributed to carbon of benzene ring. The chemical shift at 128.26 (s), 127.01 (s), 126.07 (s), 124.87 (s), 122.77 (s) ppm can be attributed to C2、C4、C7、C9、C11、C12、Cd and Cq respectively. The chemical shift at 89.32(s)ppm can be attributed to alkynyl carbon.

FAM labeled chimera were used to investigate the cellular uptake via RPN. As shown in Figure 2, the fluorescence intensity of FAM labeled siRNA and chimera by RPN delivery into SKOV3 cells is higher than that of the positive group as Lipofectamine, indicating that RPN can be used as the delivery carrier of nucleic acid chimera molecules, and the delivery efficiency is higher than Lipofectamine. Moreover, the endocytosis of chimera loaded RPN is significantly higher than that of siRNA loaded nanoparticles (Figure 2A,B), indicating that the chimera has strong targeting effects and can significantly enhance the uptake of nanoparticles by cells. The cellular uptake of chimera and siRNA by RPN in ES-2 and A2780 cells was shown in Figure S2-3.

As FACS analysis shown in Figure 3, the concentration of RPN increased, the nanoparticles internalized by three ovarian cancer cells also gradually increased when the concentration of RPN was 40 μM, the intracellular uptake efficiency of chimera/siRNA by RPN was equivalent to that of the Lipofectamine group. Moreover, the uptake rate of siRNA alone by SKOV3 cells is only 1.69%, which may be due to the degradation before siRNA uptake, indicating that RPN can not only improve the uptake efficiency of siRNA, but also protect siRNA against degradation effectively.

The toxicity of nano-vector is an important index to evaluate the vector system. An ideal transfection vector should not only have high transfection efficiency, but also have low toxicity. As a result, as shown in Figure 4A, the concentration of RPN was less than 100 μM, the cell viability of three ovarian cancer cells (SKOV3, ES-2 and A2780) was 90% or more, and the toxicity of RPN was lower than Lipofectamine 2000. It can be used as a safe carrier for chimera delivery. Only when the concentration of RPN is greater than 150 μM, much higher than general dosage of use, it gradually showed obvious cytotoxicity.

Notch3 gene has been proved to be related to the proliferation of tumor cells in previous studies. RPN-chimera complex enters the cells and down-regulates or silences the expression of Notch3 through RNA interference, so as to inhibit the proliferation of ovarian cancer cells. As shown in Figure 4B, the inhibition efficiency of RPN-chimera complex was significantly higher than that of RPN-siRNA group, indicating that the EGFR aptamer in the chimera played a certain targeting role and enhanced the inhibition efficiency. RPN also exhibited higher transfection efficiency compared with Lipofectamine for in vitro chimera delivery. As shown in Figure 4C-E, when the amount of chimera is in the range of 0-5pmol, the proliferation of ovarian cancer decreases, but when it is in the range of 5-10pmol, the proliferation of ovarian cancer does not decrease significantly, indicating that the inhibition efficiency of RPN-chimera complex against the proliferation of tumor cells is concentration dependent before saturation of chimera loading by RPN, but redundant chimera molecules can't lead to higher silencing effects.

The chimera delivery via RPN could inhibit the Notch3 expression efficiently. As shown in Figure 5A, the results of real-time fluorescence quantitative PCR showed that RPN-chimera

complex achieved the highest inhibition efficiency against Notch3, and the relative mRNA expression value was 0.3. When the RPN was not used as the carrier, the mRNA expression increased significantly. In the non-targeting RPN-siRNA group, the relative mRNA expression value was about 0.9, which was much higher than that of RPN-chimera. The mRNA inhibition rate of RPN-chimera was close to that of Lipofectamine-chimera group. The chimera played a synergistic targeting role in SKOV3, A2780 and ES-2 cells, enhancing the interference effect of Notch3 siRNA. Under the protection of RPN nano-vector, the inhibition rate of RPN-chimera complex is very high. As shown in Figure 5B, the western blot results further showed that the RNA interference effect of chimera/Notch3 siRNA against ovarian cancer cells was effective at the protein expression level.

The apoptosis was obtained by flow cytometry analysis in Figure 6. Results showed that RPN-chimera complex could induce the cell apoptosis of three kind of ovarian cancer cells significantly, compared with positive control as Lipofectamine. RPN-chimera induced the highest apoptosis rate in SKOV3 cells as 30.1%, higher than RPN-siRNA group as 19.5%. The apoptosis rate against ES-2 and A2780 cells induced by RPN-chimera complex was 19.3% and 29.8% respectively as shown Figure S4-5. RPN could induce higher apoptosis rate both by chimera and siRNA loading compared with Lipofectamine, indicating the superior delivery efficiency of RPN than Lipofectamine. On the other hand, the chimera and Notch3 siRNA alone could not induce apoptosis of cancer cells without the protection of nanoparticles.

For the in vivo antitumor study, the RPN nanoparticles were administered by intravenous injection at a dose of 5mg/kg. After injection, the body weight of nude mice generally showed an upward trend, and there was no significant difference among each group, indicating that the intravenous administration of RPN had little effects on the body weight of mice (Figure 7). In addition, the pathological sections of liver and kidney of nude mice in each group were studied by HE staining. No tumor metastasis, cell injury, inflammation and necrosis were observed in each group. It shows that the toxic and side effects of RPN-chimera administration by intravenous injection are small. After a period of administration, the size of tumor tissue in nude mice treated with RPN-chimera and Lipofectamine-chimera was significantly lower than that of normal saline group (Figure 7C,D), which significantly inhibited the tumor growth. The

tumor volume of the chimera alone group was lower than those of the normal saline group, which also showed a certain inhibitory effect on tumor growth, but the tumor inhibition rate of chimera alone was lower than RPN-chimera. The tumor inhibition rate of RPN-chimera group was 78.8% and that of Lipofectamine 2000 chimera group was 72.7%. The tumor inhibition rate was significantly higher than that of RPN-siRNA and Lipofectamine 2000 siRNA groups, indicating that the tumor inhibition effect of chimera in vivo was more obvious than that of siRNA alone. The synergistic targeting effect of aptamer and siRNA enhanced the interference effect of siRNA.

In the immunohistochemical experiment, the staining of Notch3 antigen of tumor tissue sections in RPN-chimera, Lipofectamine 2000+chimera group was weakly positive, RPN showed the highest inhibition effects against Notch3 expression in tumor tissue, compared with RPN-siRNA, Lipofectamine 2000 siRNA, chimera group and siRNA group (Figure 8). As shown in the results of western blot, the expression of Notch3 protein in nude mice administrated with RPN-chimera and Lipofectamine chimera decreased significantly, which further showed that the RNA interference effects of chimera/Notch3 siRNA was effective at the protein expression level. The expression of RPN-chimera and Lipofectamine chimera decreased more significantly, indicating that aptamer and siRNA play a synergistic targeting role and enhance the interference effects of siRNA. The therapeutic effect of nanocarrier delivery of drug-induced chimera/Notch3 siRNA was further explained.

Conclusions

In this study, the ruthenium polypyridine nanoparticles (RPN) were synthesized by chemical method as the carrier for targeted delivery of chimera, which has the characteristics of safety, low cytotoxicity and high efficiency. EGFR-Notch3 chimera was efficiently delivered via RPN to ovarian cancer cells SKOV3, A2780 and ES-2, so as to induce apoptosis, inhibit tumor cell proliferation and down regulate the expression of Notch3 mRNA and protein, chimera loaded by nanoparticles showed higher silencing efficiency than siRNA nanoparticles. EGFR aptamer and siRNA played a synergistic targeting role and enhanced the interference effect of siRNA. RPN chimera has the best effects on inhibiting tumor growth in vitro and in

vivo, and the tumor inhibition rate of RPN-chimera is higher than that of siRNA complex. This study provides a new idea for enhancing the silencing efficiency of siRNA and tumor targeting, and the chimera loaded RPN nanomaterials exhibited great promise in targeted tumor therapy as theranostic agents.

Methods

Materials

Lipofectamine2000 and DAPI staining solution were from Invitrogen, CCK-8 Kit and BCA quantitative Kit were from Sigma Aldrich. 30% acrylamide, 10% SDS, 10% ammonium persulfate, and annnexin cell apoptosis detection kit was from Bio-rad. Tetramethylethylenediamine (TEMED) was from Ameresco. Notch3 primary antibody, GAPDH primary antibody, HRP labeled anti-rabbit secondary antibody Abcam. Notch3 primer, GAPDH primer, PrimeScript™ RT Master Mix、SYBR ® Premix ex Taq II Kit were from Takara.

Cell Line and Cell Culture

SKOV3, ES-2, and A2780 cell lines （ATCC）were obtained from Shanghai Institute of Cell Biology (Shanghai, China). Fetal bovine serum (FBS), McCoy's 5A medium, and Hyclone were from Gibco. According to 90% DMEM/McCoy's 5A basic medium + 10% FBS + 100 μg/ml, complete medium was prepared, McCoy's 5A complete medium was added to SKOV3 cells and cultured in 37 ℃ 5% CO_2 for 24h, DMEM complete medium was added to ES-2 and A2780 cells and cultured at (37 ℃, 5% CO_2) for 24h.

Synthesis and Characterization of RPN

The RPN complexes were synthesized using an Anton Paar monowave 300 microwave reactor [an Initiator single-mode microwave cavity at 2450 MHz (Biotage)]. In 10 ml Pyrex reaction tube, add D-[Ru (bpy)2(p-brpip)]$(ClO_4)_2$, acetonitrile, phenylacetylene, triethylamine, Pd (PPh_3) $2Cl_2$ and CuI under the protection of argon, microwave for 15 min. After the reaction, filter and spin dry under reduced pressure. Dissolve the crude product with acetonitrile, fill the column with neutral alumina column, elute radiation with acetonitrile, collect the main red

components, the product was vacuum dried to obtain brownish red solid, which is the ruthenium polypyridine nanoparticles (RPN).

The RPN samples were placed onto copper grill covered with nitrocellulose, the grid was dried at room temperature, then negatively stained with 2% phosphotungstic acid. Transmission electron microscopy (TEM) images of RPN were acquired using a JEM-2100 TEM (JEOL, Japan). Hydrodynamic diameter and size distribution of the prepared RPN were measured by DLS using ZETASIZER Nano Series Nano-ZS ZEN3600 (Malvern Instruments Ltd., UK). UV-vis-NIR was used to detect the UV absorption spectrum of RPN; The fluorescence excitation and emission spectra of RPN were detected by fluorescence spectrophotometer. The 1H and 1H−1H COSY NMR spectra were recorded in a dimethyl-d6 sulfoxide (DMSO-d6) solution on a Bruker DRX 2500 spectrometer, and electrospray ionization mass spectrometry (ESI-MS) spectra were obtained in methanol on an Agilent 1100 ESI-MS system operating at room temperature. UV−vis absorption spectra were recorded on a Shimadzu UV-2550 spectrophotometer using 1-cm-path-length quartz cuvettes (3 mL). Circular dichroism (CD) spectra were measured on a Jasco J-810 spectropolarimeter.

Synthesis of EGFR-Notch3 chimera

The RNA aptamer sequence specifically recognized by EGFR was screened through optical imaging of quantum dots, and Genscript was entrusted to synthesize EGFR DNA aptamer[19]. EGFR DNA aptamer was transcribed into EGFR RNA aptamer; Dilute EGFR RNA aptamer tenfold and take 10 μ L EGFR RNA aptamer and 10 μ L Notch3 siRNA hybridization: react at 65 ℃ for 10min and 37 ℃ for 5min.

Notch3 siRNA sequence

Notch3 siRNA sense GUCAAUGUUCACUUCGCAGUU

Notch3 siRNA antisense AACUGCGAAGUGAACAUUGAC

NC siRNA sense UUCUCCGAACGUGUCACGUTT

NC siRNA antisense ACGUGACACGUUCGGAGAATT

Cellular Uptake

SKOV3, ES-2, and A2780 cell lines were used to investigate the uptake of RPN. 2-3×10^4 cells were inoculated in each well of 24 well plate and cultured overnight. RPN-chimera (chimera concentration gradient: 0.4, 4, 20, 40, 80μM, 1 μL/well) was incubated with cells for 4h at (37 ℃, 5% CO_2). Lipofectamine 2000 was used as the positive control group according to its instructions. After incubation, the cells were washed thoroughly with DPBS and trypsinized. Cells were then centrifuged to collect the pellet, then re-suspended in DPBS. The cellular uptake of RPN-chimera complex was analyzed by flow cytometry (FACS Canto II, Becton Dickinson, San Jose, CA, U.S.), and FITC (FL1, ex = 488 nm) labeled chimera was selected as the detection molecule. The experiment was repeated for 3 times, and the experimental results were processed and analyzed by flowjo 7.6.1 and graphpad prism 5.

The interaction of cells with RPN-chimera complex was observed and photographed using a confocal microscope.

In Vitro Cytotoxicity Assay

It is a colorimetric assay to determine the cytotoxicity of RPN by use of CCK-8 kit. Briefly, cancer cells (SKOV3, ES-2, and A2780) were seeded in 96 well tissue culture plate at a density of 10×10^3 cells per well for 24h. After 80% confluency, the cells were incubated with RPN with different (25、50、75、100、150、200、250μM) concentrations for another 24 h. The positive control group was 0.25μL/well Lipofectamine 2000 diluted with blood-free pure medium. Blank group (only complete medium without inoculated cells) and control group (with complete medium and inoculated cells) were set. After incubation with RPN and lipofectamine, the original medium of 96 well plate was discarded, washed with PBS once, then 10 μL/well CCK-8 was mixed 100 μL/well complete medium, and added to the 96 well plate. 96 well plates were incubated in a constant temperature incubator for 2-3h. Finally, the absorbance of the solution was measured at 570 nm using a Bio-Rad multimode plate reader.

In vitro RNA interference by RPN-chimera

Different doses of chimera/Notch3 siRNA were transfected by 30μM RPN into SKOV3, ES-2 and A2780 cells respectively. After incubation at room temperature for 10min, they were added to a 96 well plate containing cells and culture medium. Lipofectamine 2000 was used as the positive control group to transfect chimera/Notch3 siRNA into SKOV3 cells according to the instructions. The cells were cultured in incubator for 24-48 hours.

The cell activity was detected by CCK-8 kit. The apoptosis of SKOV3, ES-2 and A2780 cells induced by chimera/Notch3 siRNA was detected by flow cytometry. Annexin v-apc apoptosis reagent was added after cell culture with chimera/Notch3 siRNA loaded RPN. Cells were then digested and collected by trypsin, stained with annexin v-apc and detected at wavelength (ex=633nm, EM=660nm). Real-time quantitative PCR was performed by real-time quantitative PCR 7500 of ABI to detect Notch3 gene expression. Western blot was used to detect the expression of Notch3 protein after chimera/Notch3 siRNA delivery.

In Vivo Ovarian Cancer Model

Female nude mice were purchased and raised through the animal center of Tongji University. When the nude mice grew to 4-6 weeks old, SKOV3 cells (1.5×10^7) in logarithmic growth period were implanted subcutaneously in the right flank of female nude mice. The body weight of nude mice was measured every two days. The tumor volume was calculated by using the formula, V (volume)=$LW^2/2$, where "L" represents the greatest length and "W" represents the perpendicular width. When tumors reached a mean size of 100-150 mm^3, animals were randomly split into seven groups (six mice in each group) with different treatment. The drug therapy consisted of normal saline (blank control), Notch3 siRNA, EGFR-Notch3 chimera, RPN+Notch3 siRNA, RPN+chimera, Lipofectamine 2000+Notch3 siRNA and Lipofectamine 2000+chimera. The dosage of chimera or siRNA was 5 μg/20g. All the drugs were intravenously administered once every two days for 12 days. The weight of nude mice and the volume of tumor tissues were measured before each administration. The animals were sacrificed when the average tumor size of the control exceeded 6000 mm^3. The tumor tissues were harvested immediately following. Some tumor tissues were fixed in 4% paraformaldehyde for subsequent immunohistochemical detection of Notch3 expression, and some were stored in

-80 ℃ refrigerator for subsequent western blot detection of Notch3 protein expression. The liver and kidney were dissected from the mice and collected in 10% formalin for subsequent hematoxylin eosin (HE) staining. All animals procedures were approved by Institutional Use and Care of Animal Committee.

Western Blot

Frozen tumor tissues from mice in vivo treated by different drugs above were minced and homogenized in RIPA buffer (Sigma) with a protease inhibitor mix. Equal amounts of total protein lysate of tumor tissues were denatured in Laemmeli buffer at 70 ℃ for 5min, separated by SDS/PAGE and transferred onto PVDF membranes. The monoclonal antibody against Notch3 was used at a 1:1000 dilution in 5% BSA. The membranes were incubated with the primary antibody at 4 ℃ overnight, then detected with anti-rabbit secondary antibody at 1:1000 dilution for 1 h. The proteins were measured using an ECL detection system according to the manufacturer's protocol.

Immunohistochemical Staining and Histopathology Evaluation

Following formalin fixation and paraffin-embedding, the tumor tissue were incubated with the primary antibody Notch3 for 60 min, washed with PBS, and then incubated with the secondary antibody. Finally, the sections were stained with 3,3-diaminobenzidine and then counterstained with hematoxylin. Images were obtained by fluorescence microscope.

The liver and kidney were dissected from the mice for histopathological analysis. Sections were cut from the paraffin-embedded slices at 5 μm thickness, mounted on APES-coated slides, deparaffinized and stained with hemtoxylin-eosin to assess histological alterations by Nikon Eclipse TE2000 microscope.

Statistical analysis

All results represent the average of at least three separate experiments, and are expressed as mean±SD. Statistical analysis was performed using t-test. $P<0.05$ was considered statistically significant.

Author Contributions

Lifeng Qi conceived and supervised the project, analysed the data, and wrote the manuscript. Xia Li and Qiong Wu performed the experiments and analyzed the data. Wenjie Mei and Jianjun Wang mentored the performance of the experiments.

Conflicts of interest

There are no conflicts to declare.

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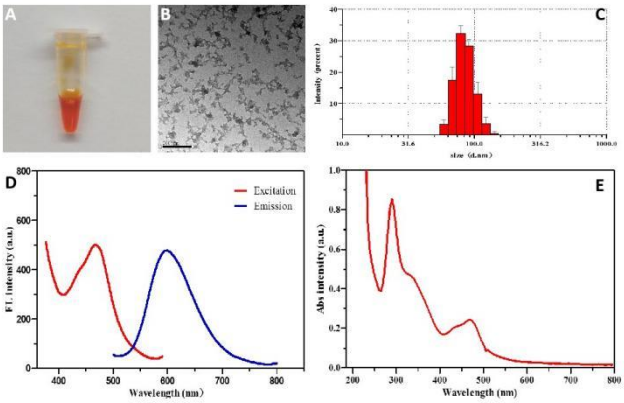

Figure 1 A: photograph of RPN nanoparticle solution (20μM), B:TEM picture of nanoparticles (bar = 500nm); C: hydraulics particle size of RPN nanoparticles; D: fluorescence excitation and emission spectrum of particles; E: UV absorption spectrum of particles.

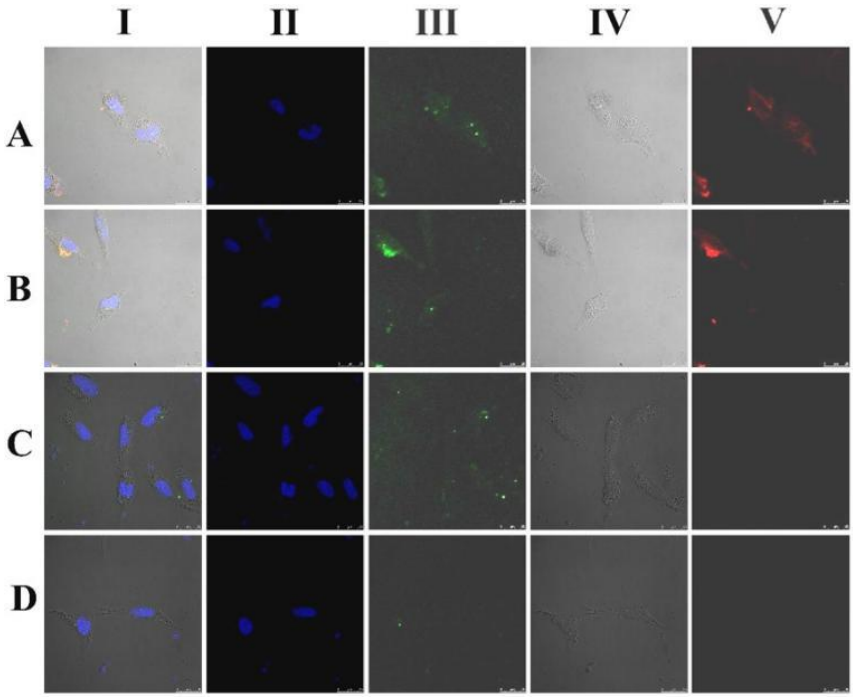

Figure 2. CLSM images of SKOV-3 ovarian cancer cells internalized with FAM labelled Notch3 siRNA and EGFR-Notch3 chimera via RPN (A： RPN+Notch3 siRNA, B: RPN + chimera, C: Lipofectamine +Notch3 siRNA, D: Lipofectamine 20000 + chimera. I: Merge, II: DAPI channel, III: FAM channel, IV: Bright field. V: Fluorescence of RPN channel.

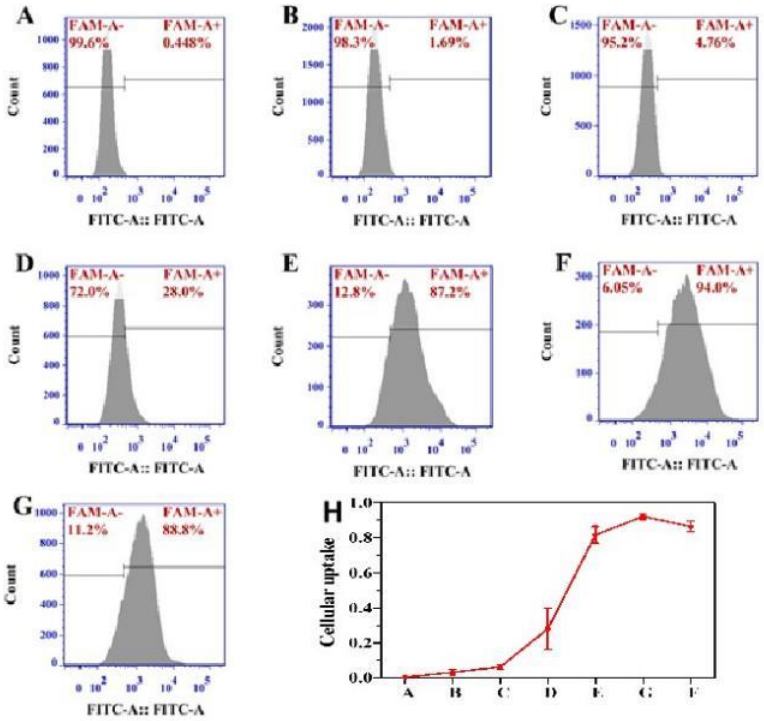

Figure 3. FCM analysis of transfection efficiency (A: PBS; B: EGFR siRNA group; C: Chimera group; D: 20 μM RNP-chimera; E: 40 μM RNP-chimera; F: 80 μM RNP-chimera; G: Lipofectamine2000+ RNP-chimera; H: Average cellular uptake efficiency by three ovarian cancer cells.

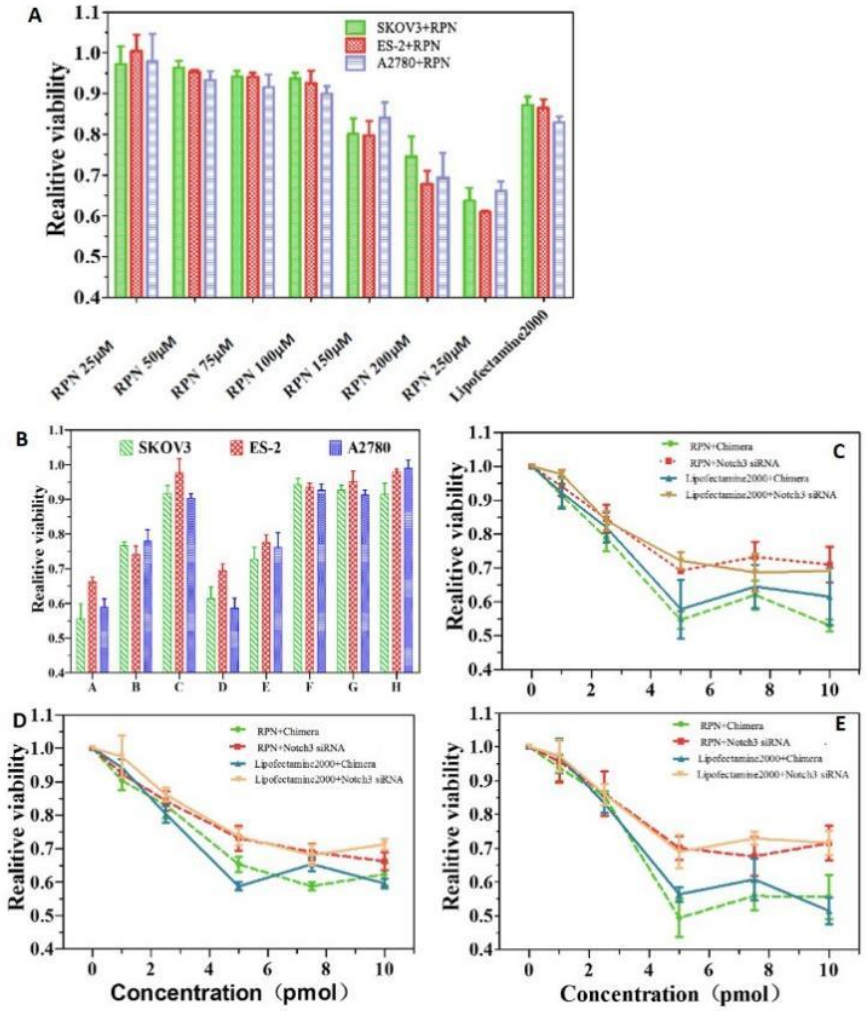

Figure 4. In vitro cytotoxicity of RPN (A) and in vitro antitumor activity of RPN-chimera against SKOV3, ES-2 and A2780 ovarian cancer cells (B), and dose dependent inhibition rate of RPN-chimera complex against ovarian cancer. C: SKOV3, D: ES-2, E: A2780 cells.

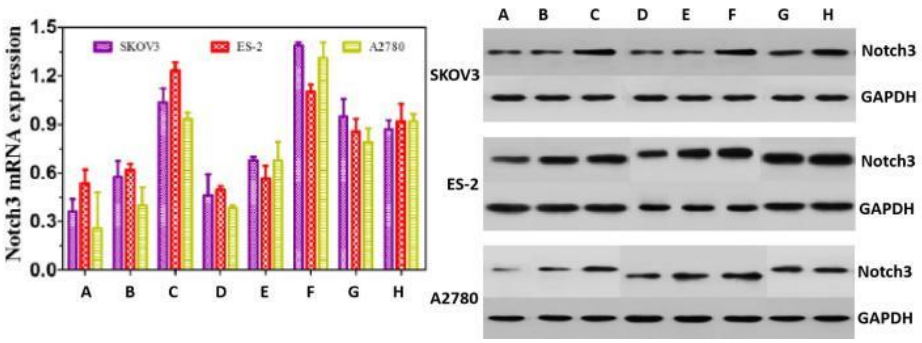

Figure 5. Notch3 mRNA and protein expression in three kind of ovarian cancer cells (SKOV3, ES-2 and A2780) after different treatments. A: RPN+ chimera, B: RPN+ Notch3 siRNA, C: RPN+ NC siRNA, D: Lipofectamine+chimera, E: Lipofectamine+Notch3 siRNA, F: lipofectamine2000+NC siRNA, G: Chimera, H: Notch3 siRNA. Corresponding western blot results of the cells treated with different drugs were shown.

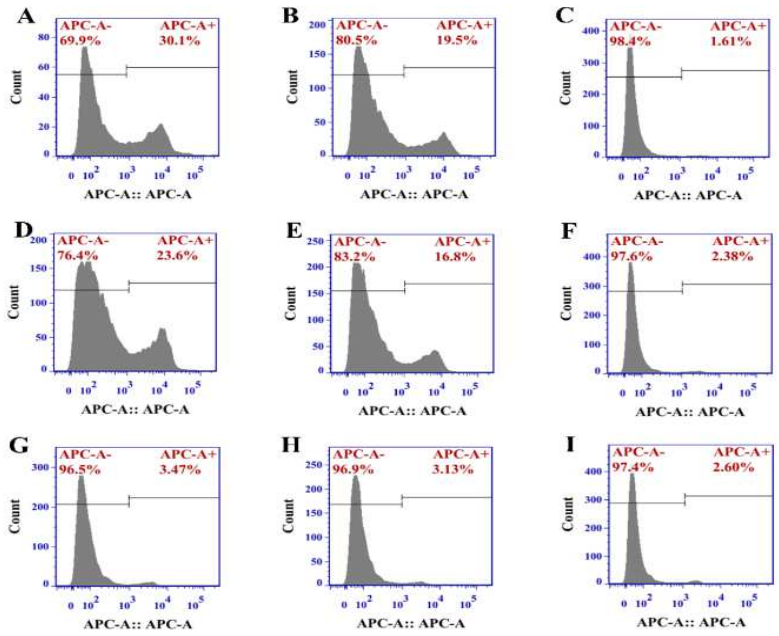

Figure 6. Apoptosis analysis by FACS in SKOV3 cells incubated with different drugs. A: RPN+chimera, B: RPN+Notch3 siRNA, C:RPN+NC siRNA, D: lipofectamine2000+chimera,E:lipofectamine2000+Notch3 siRNA, F: lipofectamine2000+ NC siRNA, G: chimera, H: Notch3 siRNA, I: normal saline.

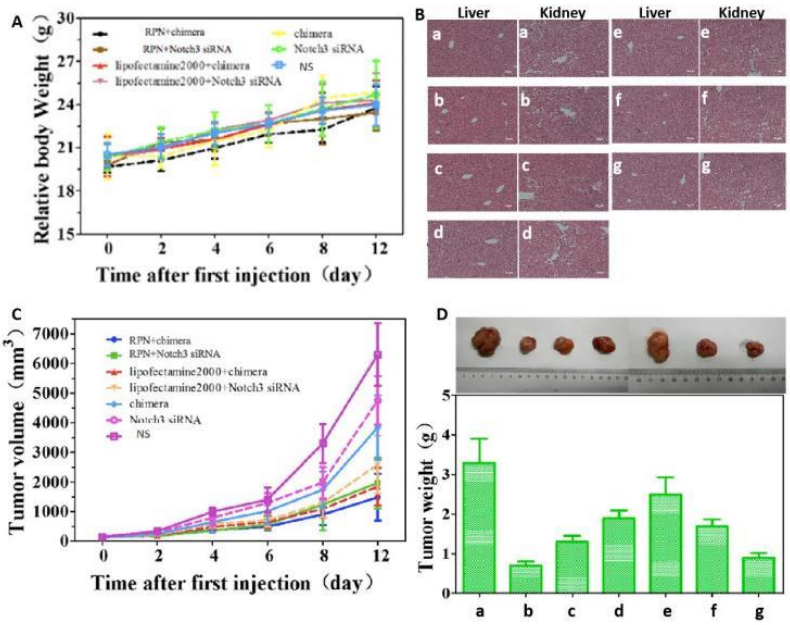

Figure 7. In vivo antitumor activity of RPN-chimera against SKOV3 ovarian cancer cells, the body weight change (A) and the H&E staining of liver and kidney tissues (B), the tumor volume change (C) and tumor weight from mice received different drug administration (a: NS: normal saline, b: RPN+ chimera, c: RPN+ Notch3 siRNA, d: chimera, e: Notch3 siRNA, f: lipofectamine2000+Notch3 siRNA, g: lipofectamine2000+chimera).

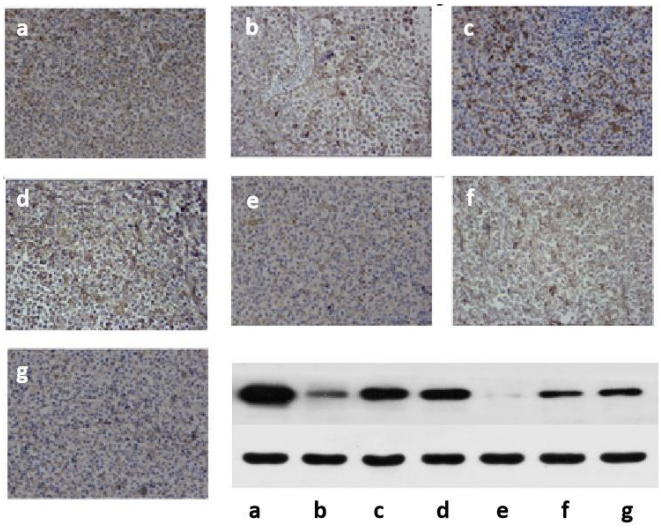

Figure 8. Notch3 expression analysis by immunohistochemical Staining and western blot in tumor tissue from from mice received different drug administration (a: NS, b: Lipofectamine2000+Notch3 siRNA, c: Notch3 siRNA, d: chimera, e: RPN+ chimera, f: RPN+ Notch3 siRNA, g: Lipofectamine2000+chimera).

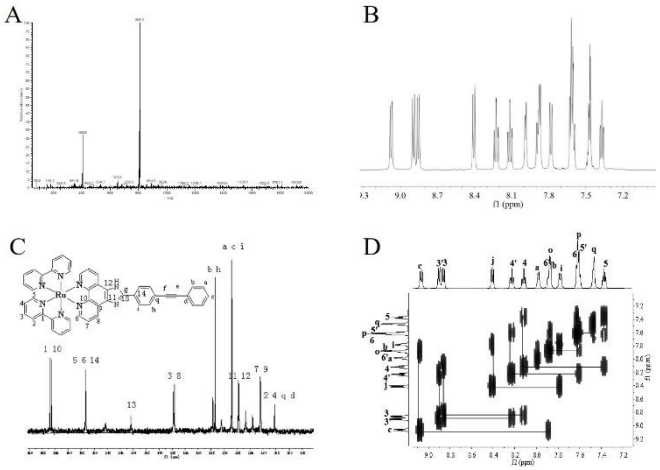

Figure S1. Characterization of Λ-RPN.(A) ESI-MS of Λ- RPN (in CH_3CN, m/z), m/z 809.3 (100%) and m/z 405.3 (33%) were attributed to $[M\text{-}2ClO_4\text{-}\ H]^+$ and $[M\text{-}2ClO_4]^{2+}$, respectively. (B) ^1H NMR spectrum (500 MHz, d^6-DMSO, ppm) of Λ- RPN. (C) ^{13}C NMR spectrum (126 MHz, d^6-DMSO, ppm) of Λ- RPN. See the inset for labeling scheme. (D) ^1H-^1H COSY spectrum (500 MHz, d^6-DMSO, ppm) of Λ- RPN.

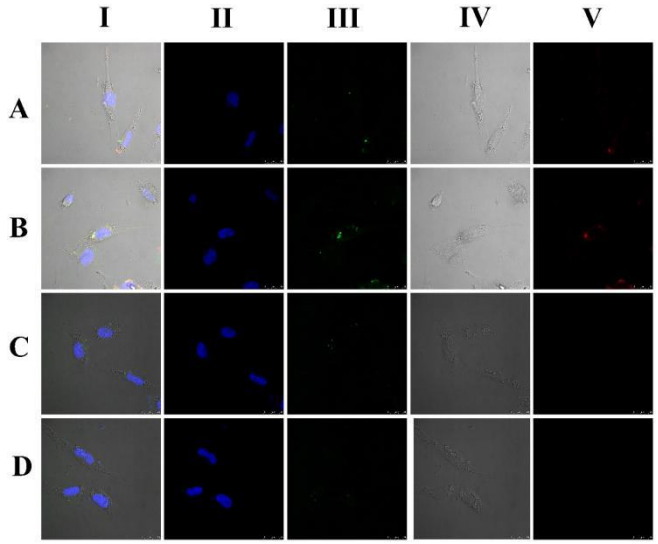

Figure S2. CLSM images of ES-2 ovarian cancer cells internalized with FAM labelled Notch3 siRNA and EGFR-Notch3 chimera via RPN (A： RPN+Notch3 siRNA, B: RPN + chimera, C: Lipofectamine +Notch3 siRNA, D: Lipofectamine 20000 + chimera. I: Merge, II: DAPI channel, III: FAM channel, IV: Bright field. V: Fluorescence of RPN channel.

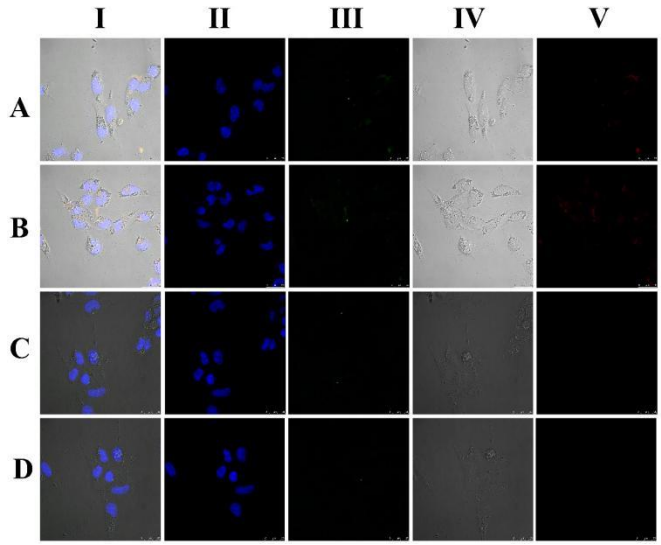

Figure S3. CLSM images of A2780 ovarian cancer cells internalized with FAM labelled Notch3 siRNA and EGFR-Notch3 chimera via RPN (A： RPN+Notch3 siRNA, B: RPN + chimera, C: Lipofectamine +Notch3 siRNA, D: Lipofectamine 20000 + chimera. I: Merge, II: DAPI channel, III: FAM channel, IV: Bright field. V: Fluorescence of RPN channel.