IGF-1 Conjugated Sub-5 nm Ultrafine Iron Oxide Nanoparticle Enhances Targeted Drug Delivery and Efficacy of DNA Topoisomerase Inhibitor SN38 for Pancreatic Cancer: An in Vitro Study

Peijia Xu  
Affiliated Longhua People's Hospital, Southern Medical University

Ting Xue  
Affiliated Longhua People's Hospital, Southern Medical University

Jonathan Padelford  
5M Biomed, LLC

Xingkui Xue  
Affiliated Longhua People's Hospital, Southern Medical University

Alyssa Y Wu  
Emory University

Yuancheng Li  
5M Biomed, LLC

Liya Wang  
Affiliated Longhua People's Hospital, Southern Medical University

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Abstract

Background

Pancreatic cancer remains one of the most lethal cancers largely due to the inefficient delivery of therapeutics. Nanomaterials have been extensively investigated as drug delivery platforms, showing improved drug pharmacodynamics and pharmacokinetics. However, their applications in pancreatic cancer have not yet been successful due to limited tumor delivery caused by dense tumor stroma and distorted tumor vasculatures. Meanwhile, smaller-sized nanomaterials have shown improved tumor delivery and retention in various tumors, including pancreatic tumors, suggesting their potential in enhancing drug delivery.

Methods

An ultrafine iron oxide nanoparticle (uIONP) was used to encapsulate 7-ethyl-10-hydroxyl camptothecin (SN38), the water-insoluble active metabolite of chemotherapy drug irinotecan for treating pancreatic cancer in clinic. Insulin-like growth factor 1 (IGF-1) was conjugated to uIONP as a ligand for targeting pancreatic cancer and stromal cells overexpressing IGF-1 receptor (IGF1R). The SN38 loading and release profile were characterized. The cancer cell targeting and induced apoptosis by developed nano-formulation IGF1-uIONP/SN38 were also investigated.

Results

IGF1-uIONP/SN38 demonstrated stable drug loading in physiological pH with the loading efficiency of 68.2 ± 3.5% (SN38/Fe, wt%) and <7% release for 24 hours. In tumor-interstitial- and lysosomal-mimicking pH (6.5 and 5.5), 52.2 and 91.3% of encapsulated SN38 were released over 24 hours. The IGF1-uIONP/SN38 exhibited specific receptor-mediated cell targeting and cytotoxicity to MiaPaCa-2 cells with IC50 of 11.8 ± 2.3 nM, but not to HEK293 human embryonic kidney cells.

Conclusion

The IGF1-uIONP significantly improved the delivery of SN38 to targeted pancreatic cancer cells, holding the potential for in vivo theranostic applications.

Introduction

Pancreatic ductal adenocarcinoma (PDAC), generally referred as pancreatic cancer, represents the majority of malignant neoplasms of the pancreas with a 5-year survival rate <7%[1]. As only 10-15% of patients are suitable for surgical resection, chemotherapy plays a critical role in the clinical management of pancreatic cancer[2]. However, gemcitabine, used as a first-line chemotherapy for pancreatic cancer for
decades[3], has not achieved satisfactory clinical outcomes, due to its poor stability in plasma, inefficient uptake by cancerous cells and deactivation by blood and hepatic cells[4]. Another first-line chemotherapy regimen consisting of 5-fluorouracil, folic acid, irinotecan and oxaliplatin, namely FOLFIRINOX, achieved better patient survival than gemcitabine, but adverse side effects were reported[4]. Nanomaterial-based drug delivery systems, at the meantime, demonstrated advantages in improving the delivery efficiency of drugs through enhanced permeability and retention (EPR) driven passive targeting and ligand-mediated active targeting to tumors, and preventing the drugs from premature release, thus reducing the systemic toxicity[5, 6]. Several US Food and Drug Administration (FDA) approved nano-formulations for pancreatic cancer, such as albumin-bound paclitaxel (nab-paclitaxel)[7] and liposomal irinotecan formulation MM-398[8], have shown increased tumor delivery of drugs and improved inhibition of tumor growth in preclinical studies[9], and more beneficial for patient survival in clinical trials[7, 10]. However, the effectiveness of nanomaterial-based formulation was impeded, largely due to the pathological barriers of pancreatic cancer. For example, the desmoplastic stroma with resulted solid stress and interstitial fluid pressure prevent the nano-formulations from reaching the cancer cells in the tumor parenchyma[11, 12]. The hypovascularity of the pancreatic tumor causes limited fenestrations of tumor blood vessels, lowering the delivery of nano-formulations to the tumor[13].

The size of nanomaterials has been shown critical for efficient tumor penetration to improve the treatment efficacy. On a mouse model of pancreatic tumor featuring extensive stroma and poor vascularization with reduced permeability, a 30 nm micelle demonstrated significantly improved tumor accumulation and anti-tumor activity comparing to the larger counterparts (up to 100 nm)[14]. Iron oxide nanoparticles (IONPs) with sizes typically ranging from 10 to 50 nm have been extensively investigated as drug delivery systems for a variety of cancers[15, 16], including pancreatic cancer[17, 18], owing to its biocompatibility, drug loading capability and capacity as imaging probes[19-23]. With the size further reduced to the sub-5 nm range, ultrafine IONP (uIONP) with a diameter of 3.5 nm demonstrated several-fold enhancement in passive targeting[16] and ligand-mediated active targeting[24] for solid tumors, comparing to the IONPs with core sizes of 20 and 30 nm. Hence, uIONP holds great potential to penetrate pancreatic tumors more easily than current larger-sized nano-formulations. In addition, the greater specific surface area of uIONPs allows for a substantially higher drug loading efficiency [25].

Here, we report the application of uIONPs with an amphiphilic polymer coating as carriers to deliver DNA topoisomerase I inhibitor 7-ethyl-10-hydroxyl camptothecin (SN38), the active metabolite of irinotecan with poor water solubility but much greater potency than irinotecan[26, 27]. Insulin-like growth factor 1 (IGF-1) was conjugated to the uIONP with SN38 loading (IGF1-uIONP/SN38) as the ligand to target the IGF-1 receptor (IGF1R), which is highly upregulated on the surface of pancreatic cancer cells and stromal components[28, 29] but not in normal pancreas[17]. The IGF1-uIONP/SN38 was characterized for the SN38 loading efficiency and release profile under different conditions. The pancreatic cancer cell targeting by IGF1-uIONP/SN38 and the induced cell apoptosis were also investigated.

**Methods**
Encapsulation of SN38 to uIONPs

SN38 (10 mg) was dissolved in DMSO (0.5 mL) before mixing with the uIONP solution in water (1 mg Fe/mL) in the weight ratio of 1:1 (Fe/SN38). The mixture was incubated for 24 hours at room temperature. PEG550 (1 mL) was then added. The mixture was further incubated for 24 hours at room temperature, and then centrifuged at 3000 rpm for 5 min to precipitate the free SN38. The supernatant was collected and centrifuged using an Amicon ultra-4 centrifugal filter unit to remove the free PEG550. The residual uIONP/SN38 was re-suspended in water, and the filtration/re-suspension cycle was repeated three times.

Loading Efficiency and Release Profile of IGF1-uIONP/SN38

The SN38 loading efficiency on uIONPs was defined as the weight% of SN38 on uIONPs by equation (1):

\[
\text{Loading Efficiency} = \frac{\text{Mass of SN38}}{\text{Mass of uIONP}} \times 100\% \quad \text{equation (1)}
\]

where the Mass of SN38 and Mass of uIONP were the amounts of SN38 and Fe in mg, respectively, in a given volume of uIONP/SN38 solution. The absorbance of SN38 at 380 nm was measured on a Genesys 50 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) for quantify SN38 following a protocol in the literature[30]. The Fe Weight\text{uIONP} was determined by a 1,10-phenanthroline colorimetric assay following the method described previously[31].

The SN38 release profile was determined by incubating uIONP/SN38 in buffered solutions of pH 5.5, 6.5 and 7.4, mimicking the lysosomal[32], tumor interstitial[33] and physiological environment, respectively. Briefly, uIONP/SN38 was incubated in acetate buffer (0.1M, pH 5.5), potassium phosphate buffer (1M, pH 6.5) and PBS (1X, pH 7.4) with the Fe concentration of 1 mg/mL for 1, 2, 4, 8 and 24 hours. The samples were then centrifugation at 3000 rpm for 10 min to allow the released SN38 to precipitate. The supernatant was then collected to measure the loading efficiency of SN38 using the method described above. Each measurement was repeated three times.

Conjugation of IGF-1 and NIR830 Dye to uIONPs

uIONP/SN38 was firstly conjugated with IGF-1 through sulfo-SMCC following the established procedure[34]. uIONP/SN38 (2 mg Fe/mL, 0.5 mL) was mixed with PBS (0.5 mL). Sulfo-SMCC (1 mg) was then added to incubate with uIONP/SN38 for two hours at room temperature to allow the reaction with amine groups on the surface of uIONP. Afterwards, un-reacted sulfo-SMCC was removed by a PD-10 desalting column. To the collected SMCC-uIONP/SN38, IGF-1 was added in the molar ratio of 20:1 (IGF-1/uIONP). The solution was incubated at room temperature for one hour to allow the coupling of IGF-1. The NIR830 dye was made as a NHS ester (NHS-NIR830), which readily reacted with amine groups on the surface of uIONP, following the method in the literature with IR-783 as the starting material[35]. For the coupling reaction, NHS-NIR830 was added to the IGF1-uIONP/SN38 in the molar ratio of 20:1
(NIR830/uIONP). The mixture was incubated for two hours at room temperature, before removing free NHS-NIR830 by a PD-10 desalting column to yield NIR830-IGF1-ulONP/SN38. The hydrodynamic diameters and zeta-potentials of ulONP/SN38 before and after the functionalizations were measured on a Malvern Zetasizer NanoZS90 (Malvern, United Kingdom).

**In Vitro Cancer Cell Targeting of IGF1-ulONP/SN38**

In an 8-well chamber slide, $5 \times 10^4$ MiaPaCa-2 and HEK293 cells (Procell Life Science&Technology Co., Ltd., Wuhan, China) were seeded and cultured for 24 hours in DMEM and EMEM, respectively, supplemented with 1% penicillin-streptomycin and 10% FBS. The cells were then incubated with NIR830-IGF1-ulONP/SN38 and NIR830-ulONP/SN38 for three hours at 37 ºC with the Fe concentration of 0.02 mg/mL. Afterwards, the cells were washed with PBS for three times, and fixed with 4% paraformaldehyde. After washing with PBS three times again, the cells were mounted with ProLong™ Gold Antifade Mountant with DAPI, before fluorescence imaging on a fluorescent microscope (BZ-X710, Keyence, Osaka, Japan) with a customized filter for NIR830.

**Cytotoxicity Assays of IGF1-ulONP/SN38**

The cytotoxicity assays of IGF1-ulONP/SN38, ulONP/SN38 and free SN38 for MiaPaCa-2 and HEK293 cells was performed using an AlamarBlue assay kit following the manufacturer’s protocol. In a 96-well plate, 2000 cells/well were seeded and cultured for 24 hours to allow the attachment of cells. IGF1-ulONP/SN38, ulONP/SN38 and free SN38 (20 mM dissolved in DMSO) were added to each well to incubate with the cells at 37 ºC for 72 hours with the SN38 concentrations of 0, 2.5, 4.9, 9.8, 19.5, 39.1, 78.1, 156.2, 312.5, 625, 1250 and 2500 nM. After three washes with cold PBS, the cells were measured for viability using AlamarBlue assay. Results were normalized according to the cells treated with no SN38. Data was processed using Origin software (Originlab Corporation, Northampton, MA, USA).

**Induction of Cell Apoptosis by IGF1-ulONP/SN38**

On 6-well plates, $10^5$ MiaPaCa-2 cells were seeded in each well and cultured in 1 mL DMEM supplemented with 1% penicillin-streptomycin and 10% FBS for 24 hours at 37 ºC to allow the cells to attach. Afterwards, the culture media were replaced with fresh media containing IGF1-ulONP/SN38, ulONP/SN38 and free SN38 at the SN38 dosages of 1, 5 and 10 µM. Cells treated with medium only were used as the control. The cells were incubated for additional 24 hours, before removing from the wells by trypsin-EDTA. The removed cells were collected and washed three times with PBS, and stained for annexin V and 7-AAD using the Annexin V Apoptosis kit. The cells were analyzed using a BD FACSymphony A3 flow cytometer. Measurements were repeated three times for $1 \times 10^5$ cells each time. The data was processed by Tree Star FlowJo software (FlowJo, LLC, Ashland, OR, USA).

**Statistical Analysis**

Data was presented in the format of mean ± standard deviation. Statistical significance was determined by a two-tailed unpaired t test when comparing the hydrodynamic diameters and zeta-potentials of
uIONPs before and after SN38 loading and conjugation of IGF-1. The level of significance was set at $p < 0.05$.

**Results**

**Characterization of SN38 Loaded uIONPs**

The commercial amphiphilic polymer coated uIONPs with an averaged diameter of 3.5 nm was used for encapsulating SN38. The uIONP demonstrated a narrow distribution of hydrodynamic diameter measured by dynamic light scattering (Fig. 1a), indicating monodispersed uIONPs with a highly uniformed size, which was further supported by the transmission electron microscopic (TEM) image (Fig. 1a, inset). After mixing SN38 with uIONPs to allow the accumulation of SN38 in the hydrophobic moiety of the coating polymer, PEG550 was added to further stabilize the loading of SN38. The emerging peak at ~ 380 nm for uIONP/SN38 in the UV-Vis spectrum, comparing to that of uIONP alone and free SN38 peaked at ~ 405 nm (Fig. 1b), indicated the loading of SN38 to the amphiphilic polymer coated uIONPs[30]. Worth noting, the peak of SN38 blue shifted after encapsulation, possibly due to the ionic hydrogen bonding between the Fe and SN38 molecules[36].

The loading efficiency of SN38 was measured to be $68.2 \pm 3.5\%$ and found stable in PBS (pH 7.4), with a <7% variation over 24 hours (Fig. 1c), suggesting the capability of uIONP/SN38 in preventing the premature drug release during blood circulation. At pH of 6.5 and 5.5, which mimicked the acidic conditions in tumor interstitium[33] and lysosome[32], 47.8% (pH 6.5) and 9.7% (pH 5.5) of SN38 remained encapsulated over 24 hours (Fig. 1c), indicating the potential environment-triggered drug release in the tumor interstitial space and the lysosomes of cancer cells after IGF1-uIONP/SN38 was internalized through receptor-mediated endocytosis[37]. The release profiles of uIONP/SN38 showed a plateau-like stages from one to two hours after exposing to the acidic solutions, indicating a stepwise SN38 release. As the addition of PEG550 further increased the SN38 loading efficiency comparing to that without PEG550 (48.3 ± 6.1%), which showed no plateau-like stage in the release profile (Supporting Information, Fig. s1), the release pattern in Fig. 1c can be ascribed to the fast release of PEG550 with the loosely encapsulated SN38 molecules within one hour, and a subsequent release of SN38 from the inner layer of coating polymer.

**Optimization and Functionalization of uIONP/SN38**

To investigate the effect of PEG molecules in stabilizing the SN38 loading, PEG with different molecular weights were tested in SN38 encapsulation. The loading efficiencies of SN38 decreased from $68.2 \pm 3.5\%$ to $42.1 \pm 3.8\%$, as the molecular weights of PEG increased from 550 to 5000 g/mol (Fig. 2a), indicating a better SN38 loading by the PEG with a lower molecular weight. With PEG550 selected for optimal loading of SN38, the encapsulation of SN38 did not result in a significant ($p > 0.05$) change on the hydrodynamic diameter (Fig. 2b) or the zeta-potential (Fig. 2c). The uIONP/SN38 was then functionalized with IGF-1 as the targeting ligand for IGF1R, whose expression was highly upregulated in both pancreatic cancer cells and stromal component. The significantly ($p < 0.001$) increased hydrodynamic diameters of uIONP/SN38
(9.9 ± 1.8 nm) to IGF-uIONP/SN38 (14.5 ± 2.1 nm) indicated the conjugation of IGF-1 (Fig. 2b), which was further confirmed by the change of zeta-potentials from the positive surface charge for uIONP/SN38 (0.99 ± 0.31 mV) to the negative charge (-0.88 ± 0.46 mV) after conjugation of IGF-1 (Fig. 2c).

**Targeting of Pancreatic Cancer Cells by IGF1-uIONP/SN38**

To validate the targeting effect of IGF1-uIONP/SN38 to pancreatic cancer cells, near infrared (NIR) dye NIR830 labeled IGF1-uIONP/SN38 was incubated with MiaPaCa-2 human pancreatic cancer cells with overexpression of IGF1R[28, 29], with HEK293 human embryonic kidney cells with normal expression of IGF1R[17] as the control cell line. NIR830-IGF1-uIONP/SN38 at the Fe concentration of 0.02 mg/mL was applied to demonstrate the difference in cellular uptake of NIR830-IGF1-uIONP/SN38 by MiaPaCa-2 and HEK293 cells. Extensive NIR signal was observed on MiaPaCa-2 cells (Fig. 3a to c), indicating a substantial uptake of NIR830-IGF1-uIONP/SN38 by the cancer cells. In contrast, NIR830-IGF1-uIONP/SN38 showed undetectable NIR signal on HEK293 cells (Fig. 3d to f). The IGF1-mediated pancreatic cancer cell targeting was further validated by incubating MiaPaCa-2 cells with non-targeted uIONP/SN38, in which no MiaPaCa-2 cell targeting was observed for NIR830-uIONP/SN38 when IGF-1 was not conjugated as the targeting ligand (Fig. 3g to i).

**Cytotoxicity of IGF1-uIONP/SN38**

The cytotoxicity of IGF1-uIONP/SN38 for targeted pancreatic cancer cells was investigated by measuring the viability of MiaPaCa-2 cells after incubating with IGF1-uIONP/SN38 for 72 hours at 37 °C with the SN38 concentration ranging from 0 to 2500 nM. IGF1-uIONP/SN38 demonstrated a greater inhibitory effect on the growth of MiaPaCa-2 cell than those treated with free SN38 and non-targeted treatment uIONP/SN38, which showed little toxicity (Fig. 4a). The absolute half-maximal inhibitory concentration (IC$_{50}$) of IGF1-uIONP/SN38 for MiaPaCa-2 cells was calculated to be 11.8 ± 2.3 nM (Fig. 4a). In comparison, MiaPaCa-2 treated with free SN38 reached a viability of ~ 50% at 78.1 nM with no further inhibition on cell growth at higher SN38 concentration (Fig. 4a), suggesting the limit of cytotoxicity induced by free SN38 under the experimental condition. As ~20% cells remained viable after treating with IGF1-uIONP/SN38 at the SN38 concentration of 39.1 to 2500 nM, it can be concluded that IGF1-uIONP as a drug carrier improved the delivery efficiency of SN38 to the pancreatic cancer cells than the free drug, and subsequently enhanced the efficacy of SN38 in inhibiting the cell growth. Meanwhile, IGF1-uIONP/SN38 did not exhibit obvious cytotoxicity for HEK293 cells at most SN38 concentrations, with ~10% reduction of cell viability at the concentration of 2500 nM (Fig. 4b), suggesting the biosafety of IGF1-uIONP/SN38 for non-targeted cells in healthy organs.

**Enhancement of Pancreatic Cancer Cell Apoptosis by IGF1-uIONP/SN38**

To quantitatively assess the SN38 induced cell apoptosis after the enhanced cancer cell delivery by IGF1-uIONP, IGF1-uIONP/SN38 treated MiaPaCa-2 cells were analyzed by fluorescence assisted cell sorting (FACS) after the fluorescence labeling using an Annexin V Apoptosis kit. After incubating with IGF1-uIONP/SN38 for 24 hours with the SN38 concentrations of 1, 5 and 10 µM, the MiaPaCa-2 cells did not
show a dose-dependent change on the populations of dead (AnnexinV+/7-AAD+), apoptotic (AnnexinV+/7-AAD-) and live (AnnexinV-/7-AAD-) cells, with the population of dead cells varying from 80.2 ± 0.9% to 82.2 ± 1.5% (Fig. 5a to d). Meanwhile, MiaPaCa-2 cells treated with free SN38 under the same condition exhibited increased populations of dead cells from 41.8 ± 1.2% to 53.0 ± 1.4% and decreased live cell population from 31.7 ± 1.0% to 23.9 ± 1.2%, when SN38 concentration was increased from 1 to 10 µM (Fig. 5a, e to g). These results suggested that the dosage for IGF1-uIONP/SN38 to be effective in inducing cell apoptosis was remarkably lower than that for free SN38, and IGF1-uIONP/SN38 possessed an approximate 1-fold better efficacy than free SN38 under the same experimental condition. Worth noting, although non-targeted uIONP/SN38 did not exhibit obvious cytotoxicity with the live cell population ranging from 72.5 ± 2.2% to 87.1 ± 1.3% (Fig. 5a, h to j), which was in accordance with the results in cell targeting experiment and cytotoxicity measurement, the dose-dependent decrease of live cell population suggested a minimal apoptotic effect on the cells under current condition, providing a reference for the dosing for future in vivo study.

Discussion

As one of the deadliest cancers, pancreatic cancer has been subject to limited regimen options with unsatisfying clinical outcome. The DNA topoisomerase inhibitor irinotecan, one of the major components in FOLFIRINOX, has attracted increasing attention in treating pancreatic cancer. Irinotecan has been shown to converted to its active metabolite SN38 through hydrolysis by carboxyl esterase[38], which induces apoptosis in cancer cells. However, the efficiency of irinotecan converting to SN38 is typically low (< 3%)[39]. With the attempts to directly deliver SN38 to solid tumors, various nano-formulations of SN38 have been reported in preclinical[40-42] and clinical studies[43]. Nanomaterials with smaller size has been shown to achieve better tumor penetration and accumulation than the larger counterparts in mouse model of pancreatic cancer[14]. Thus, the uIONPs with 3.5 nm as a drug carrier for SN38 hold great potential in improving the drug delivery efficiency comparing to the current SN38 nano-formulations. Moreover, the unique T₁-T₂ switchable MR contrast enhancement by uIONP based on its dispersion status[16, 44] allows for a more informative monitoring of the delivery of theranostic uIONPs to pancreatic tumors, with mono-dispersed uIONPs in tumor blood vessels showing bright T₁-weighted contrast and aggregates of uIONPs delivered to the tumor exhibiting darkening T₂-weight enhancement. Furthermore, uIONPs have shown capable in renal clearance and faster degradations in organs of the reticuloendothelial system (RES) than larger IONPs[16], suggesting an improved biosafety profile in vivo. As the prolonged tissue retention of nanomaterials, particularly the metallic compartments, raised concerns for cytotoxicity[45], the uIONPs with faster body clearance may be more suitable for cancer therapy, allowing repeated dosing while causing less systemic toxicity and side effects, comparing to the larger counterparts.

One challenge in the drug delivery for pancreatic tumors is the dense stroma that prevents the penetration of nanomaterials to the tumor, making the tumor-cell-targeted ligand on the surface of nanomaterials less effective. As the IGF1R signaling pathway plays a critical role in cell proliferation and survival[46, 47],
IGF1R is highly expressed by both pancreatic cancer cells and the stromal component[28, 29], making IGF-1 a suitable targeting ligand for pancreatic cancer. Moreover, the further elevated expression level of IGF1R on the tumor cells developing drug resistance[48, 49] makes the IGF1-uIONP a capable delivery system for delivering drug to those cells. However, IGF1R expression in human is ubiquitous[50], although not overexpressed in normal tissues[17]. Thus, the dosing of IGF1-uIONP/SN38 in future in vivo study needs to be optimized to ensure the delivery of sufficient drugs to the pancreatic tumors while minimizing the systemic cytotoxicity due to the over accumulation of IGF1-uIONP/SN38 in healthy organs resulted from IGF1R targeting.

**Conclusion**

In this study, we used a sub-5 nm uIONP with amphiphilic polymer coating as the carrier to encapsulation highly potent yet hydrophobic DNA Topoisomerase I inhibitor SN38, with low molecular weight PEG facilitating the drug loading. The resultant uIONP/SN38 demonstrated highly stable drug loading and triggered release in tumor interstitial and lysosomal mimicking acidic environment. With IGF-1 conjugated as the ligand, the IGF1-uIONP drug delivery system for SN38 exhibited targeting specificity to the pancreatic cancer cells with overexpression of IGF1R, and improved inhibitory effect on pancreatic cancer cell growth over the free drug SN38, demonstrating its potential in treating pancreatic cancer.

**Declarations**

**Ethics approval and consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Availability of data and materials** All data and materials in current study are available from the corresponding authors on reasonable request.

**Competing Interests** The authors have no relevant financial or non-financial interests to disclose.

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**Author Contributions** Liya Wang and Yuancheng Li contributed to the study conception and design. Material preparation, data collection and analysis were performed by Peijia Xu, Ting Xue, Jonathan Padelford, Alyssa Y Wu, and Xingkui Xue. The first draft of the manuscript was written by Peijia Xu and Ting Xue, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Compliance with Ethical Standards**
Disclosure of potential conflicts of interest All authors have no relevant financial or non-financial interests to disclose.

Research involving Human Participants and/or Animals Not applicable. This study does not involve any human participants or animals.

Informed consent Not applicable.

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Figures

Figure 1

(a) Hydrodynamic diameter of amphiphilic polymer coated uIONPs with a narrow distribution and the transmission electron microscopic image (inset). (b) UV-Vis spectroscopy of uIONPs (black trace), free SN38 (red trace) and uIONP/SN38 (blue trace), with the absorbance at 380 nm linearly correlated with the amount of uIONP/SN38 (inset). (c) Release of SN38 from uIONP/SN38 at the pH of 5.5, 6.5 and 7.4 within 24 hours.

Figure 2

(a) Change of SN38 loading efficiencies on uIONPs in respect to the PEG of different molecular weights used for drug encapsulation. The change of (b) hydrodynamic diameters and (c) zeta-potentials of uIONPs before and after encapsulation of SN38 with PEG550 and conjugation of IGF-1.
Figure 3

Confocal fluorescence images of (a) to (c) MiaPaCa-2 human pancreatic cancer cells with overexpression of IGF1R and (d) to (f) HEK293 human embryonic kidney cells with normal expression of IGF1R after incubating with NIR830-labeled IGF1-uIONP/SN38. (g) to (i) confocal fluorescence images of MiaPaCa-2 cells after the incubation with non-targeted NIR830-uIONP/SN38.

Figure 4

AlamarBlue assay measured viability of (a) MiaPaCa-2 human pancreatic cancer cells and (b) HEK293 human embryonic kidney cells after treating with IGF1-uIONP/SN38, uIONP/SN38 and free SN38 at 37 °C for 72 hours with the SN38 concentrations ranging from 0 to 2500 nM.

Figure 5

(a) Analysis of populations of live, apoptotic and dead cells after MiaPaCa-2 human pancreatic cancer cells were treated with IGF1-uIONP/SN38, uIONP/SN38 and free SN38. Representative flow cytometry analysis of gated cells stained by Annexin V FITC and 7-AAD showed the populations corresponding to viable and non-apoptotic (AnnexinV⁻/7-AAD⁻), apoptotic (AnnexinV⁺/7-AAD⁻) and dead (AnnexinV⁺/7-AAD⁺) cells after incubating MiaPaCa-2 cells with (b) to (d) IGF1-uIONP/SN38, (e) to (g) free SN38 and (h) to (j) uIONP/SN38.

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