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14. ABSTRACT During the no-cost extension period, we tried to resolve the problems we were having with attempting to encapsulate NZ51 in various copolymers to facilitate faster release in mouse plasma. Even the use of PLGA or chitosan derivatives did not resolve the problems. Currently, we are postulating that the chemical structure of NZ51 is interfering with the formulation and utility for in vivo experiments. Subsequently, we attempted to encapsulate a different DDX3 inhibitor into PLGA nanoparticle. This appears to be more feasible than NZ51. The manuscript describing the formulation, release kinetics and cytotoxic effects using a different DDX3 inhibitor is in preparation. In addition, we have completed the study with NZ51 as well (in preparation), which indicated that NZ51 although very efficient in killing breast cancer cells in vitro was far less efficient in controlling tumor growth in a preclinical model of breast cancer.					
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Introduction

Understanding tumor invasion and metastasis provides crucial information with respect to carcinoma progression. Towards this goal, we have identified a member of the RNA helicase family, *DDX3*, which is over-expressed in high-grade invasive breast carcinomas and induces an epithelial mesenchymal-like transformation with increased motility and invasive properties. More importantly, decreasing *DDX3* expression by shRNA reduced the metastatic load in a preclinical breast cancer model. Based on this crucial finding, we evaluated the efficacy of a novel *DDX3* inhibitor, NZ51, to potentiate cell death or reduce proliferation in breast cancer cell lines (MCF-7, MDA-MB-468 and MDA-MB-231) but not in normal immortalized breast cell lines (MCF 10A and MCF 12A). The NZ51 was designed using rational molecular modeling approach to bind to the nucleotide binding site within the *DDX3* protein molecule and abrogate its function. Preliminary data strongly indicates that NZ51 is able to selectively induce cell death in the panel of breast cancer cell lines used and not in normal immortalized breast cell lines. The above results demonstrate that abrogating *DDX3* functions in breast cancer cell lines, irrespective of ER status, can promote cell death-a mechanism that can be targeted to overcome treatment inadequacies for aggressive breast cancer such as that of the triple negative phenotype.

In this study, we proposed to study the therapeutic impact of NZ51, both in the native form as well as in nanoparticles containing dual-MR contrast agent, on the primary orthotopic tumor in a preclinical breast cancer model using non-invasive magnetic imaging techniques. Our ultimate goal is to provide targeted therapy for aggressive breast cancer phenotypes with longer disease free survival period and a better quality of life.

Body

Task 1: Generating NZ51-loaded PLGA nanoparticles

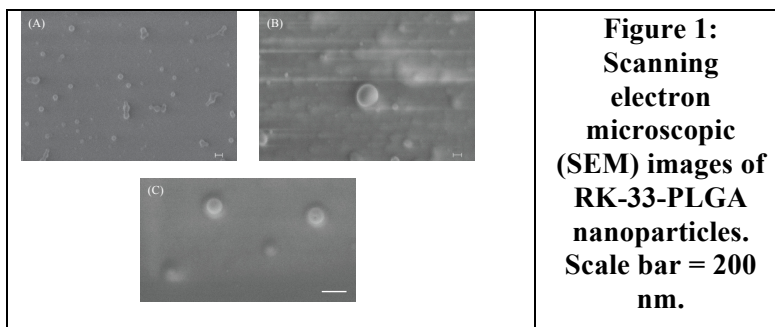
Despite numerous attempts to encapsulate NZ51 in different nanoparticles, we were unable to increase the release rate of NZ51 in mouse plasma. We are of the opinion that the chemical structure of NZ51 is probably interfering with the formulation and its utility for *in vivo* experiments. We have now continued to explore the encapsulation of a different *DDX3* inhibitor (RK-33) into PLGA nanoparticle.

Task 2: Characteristics of RK-33-loaded nanoparticles

The physicochemical characteristics of RK-33-loaded PLGA nanoparticles are listed in **Table 1**. Based on SEM, the nanoparticles are spherical in shape, and the size is consistent with DLS measurement (**Figure 1**).

Table 1. Physicochemical characteristics of PLGA nanoparticles loaded with or without a novel RNA helicase inhibitors prepared by a solvent evaporation method.

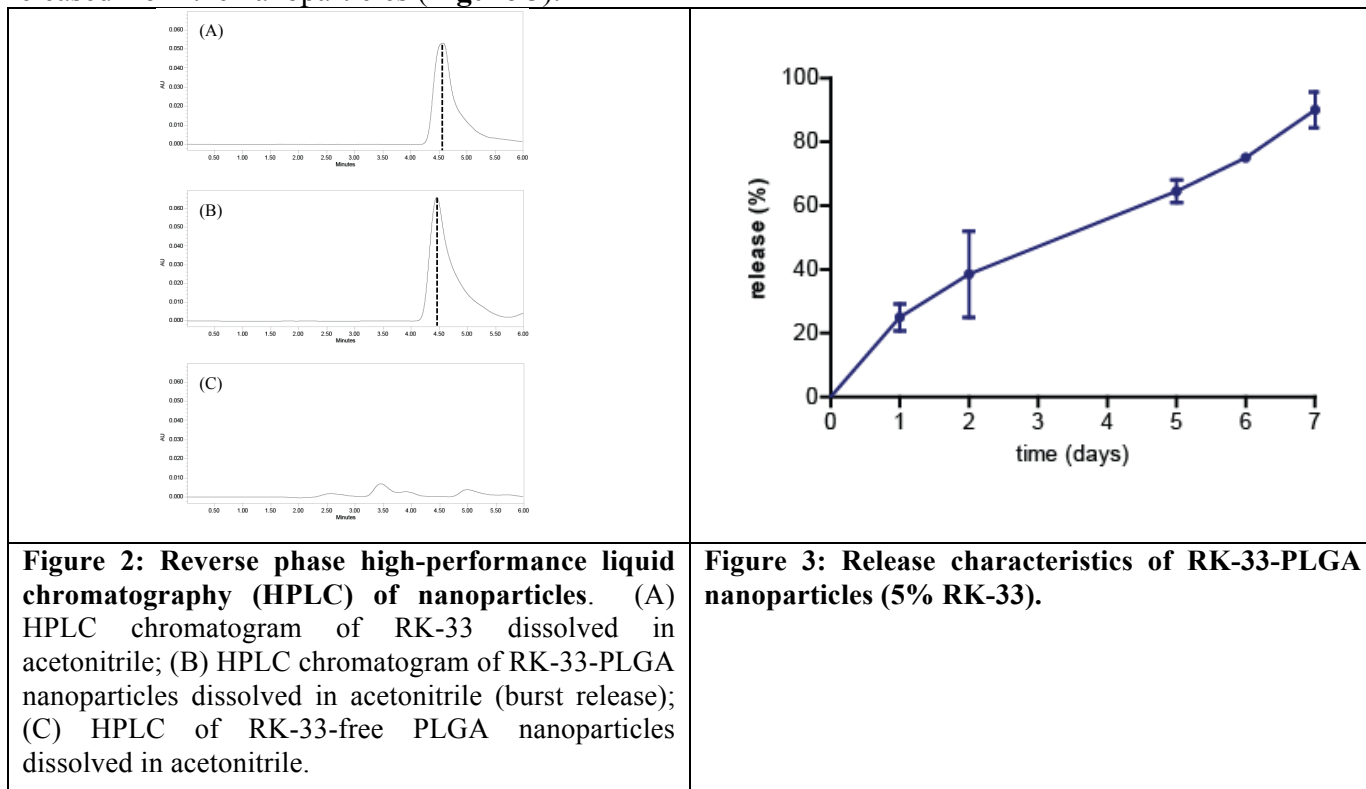
Samples	Hydrodynamic diameter $\pm$ SD (nm)	Polydispersity index	ζ -potential $\pm$ SD (mV)
PLGA	220 $\pm$ 25	0.140 $\pm$ 0.08	-2.37 $\pm$ 0.7
RK-33 PLGA (5%)	245 $\pm$ 37	0.167 $\pm$ 0.08	-2.17 $\pm$ 0.3
RK-33 PLGA (10%)	266 $\pm$ 28	0.203 $\pm$ 0.04	-2.17 $\pm$ 0.9



Task 3: Release kinetics of RK-33 loaded nanoparticles

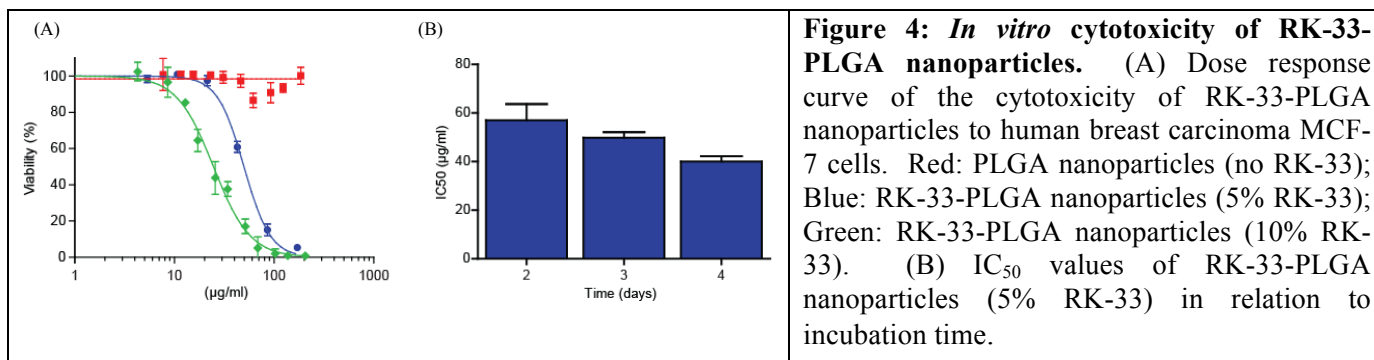
HPLC chromatogram of RK-33 showed a distinct peak at 4.5 minutes retention time, indicating that RK-33 molecules remained intact after encapsulation and subsequent burst release from these nanoparticles {**Figure 2(A) and 2(B)**}. Furthermore, breakdown products of the nanoparticle such as lactic acid and glycolic acid did not interfere with HPLC measurements {**Figure 2(C)**}.

The release of two different batches of RK-33 loaded nanoparticles was assessed in siliconized tubes, to maximize recover following release. An initial release in the first 24 hours ($25\% \pm 4.2$) was followed by a linear release of RK-33 from the nanoparticles. In seven days, $90\% \pm 5.7\%$ of RK-33 was released from the nanoparticles (**Figure 3**).



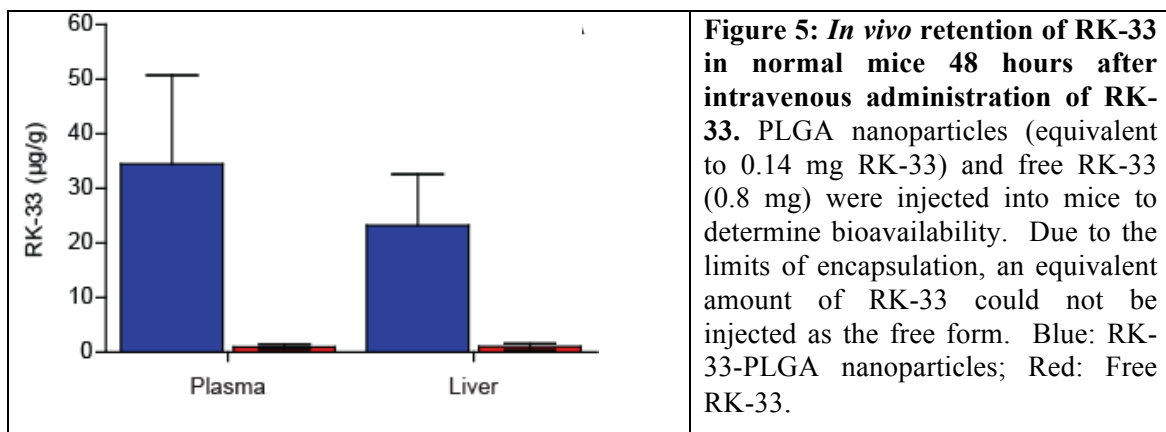
Task 4: Cytotoxicity of RK-33-loaded nanoparticles

RK-33-loaded nanoparticles demonstrated cytotoxicity to human breast carcinoma MCF-7 cells in a dose-dependent manner, whilst equivalent dose of empty nanoparticles did not {**Figure 4(A)**}. Next, we assessed whether RK-33 nanoparticles (5%) were cytotoxic to MCF-7 breast cancer cells in a time-dependent manner. The IC_{50} value of the RK-33-loaded nanoparticles was $57 \mu\text{g/mL}$ after two-day incubation, further decreasing to $40 \mu\text{g/mL}$ following four-day incubation {**Figure 4(B)**}. This indicates that RK-33-PLGA nanoparticles require higher concentration to kill MCF-7 cells compared to free RK-33 (IC_{50} : $1 \mu\text{g/mL}$) probably due to the slow sustained release formulation.



Task 5: In vivo retention of RK-33

In mice treated with free RK-33, we could not detect any RK-33 ($<1 \mu\text{g/ml}$) in the plasma, lung, or liver 48 hours after treatment. In contrast, 48 hours after injection of RK-33-PLGA nanoparticles into the tail vein of mice, we could detect RK-33 in the plasma ($34 \mu\text{g/ml}$) and liver ($28 \mu\text{g/g}$) (**Figure 5**). Although the mice treated with nanoparticles received 6 times less RK-33 dose (equivalent to 0.14 mg of RK-33) compared to the control mice (0.8 mg RK-33) due to technical limitations, RK-33 was still detected in plasma and liver indicating a steady rate release of RK-33 from the nanoparticle.



Key research accomplishments

- 1) Generated and characterized PLGA nanoparticles loaded with RK-33.
- 2) Determined that the generated RK-33-loaded PLGA nanoparticles have a hydrodynamic diameter of about 220 nm and with a slightly negative surface charge ($\approx -2.17 \text{ mV}$).
- 3) Release kinetics experiments indicate that RK-33 has a linear release rate over seven days.
- 4) RK-33 loaded nanoparticles exhibit cytotoxic effects on breast cancer cell line.

Reportable outcome

None

Conclusions

Taken together, we can report that the PLGA nanoparticle formulation of RK-33 exhibits steady state release kinetics of RK-33 in a linear manner over seven days. Moreover the RK-33 loaded nanoparticles demonstrated cytotoxic effects *in vitro*, indicating the retention of the functional activity of RK-33 following the formulation process. Furthermore, *in vivo* pilot study revealed longer systemic retention of RK-33 compared to free RK-33. This could indicate the potential use of PLGA nanoparticles as a carrier of RK-33 for clinical use.