SUMMARY

[0012] Disclosed is a composition comprising (a) a plurality of intact minicells, each minicell of the plurality encompassing plasmid-free functional nucleic acid, and (b) a pharmaceutically acceptable carrier therefor. The category of "functional nucleic acid" is illustrated by single-, double-, or multi-stranded DNA or RNA. In one embodiment, minicells of the plurality contain plasmid-free functional nucleic acid that is regulatory RNA. Examples of such regulatory RNA include, but are not limited to,siRNA, miRNA, and shRNA.

[0012a] In one aspect, there is provided a composition comprising: (a) a plurality of intact minicells, each minicell of the plurality comprising plasmid-free functional nucleic acid, and (b) at least one pharmaceutically acceptable carrier, wherein the plurality comprises a therapeutically effective amount of the functional nucleic acid.

[0013] The minicell-packaged functional nucleic acid may target the RNA transcripts encoding a protein that contributes to drug resistance, to apoptosis resistance, or to neoplasticity, *inter alia*. Also, a composition of the invention may further comprise a bispecific ligand comprised, for example, of a first arm specific for a minicell surface structure and a second arm specific for a non-phagocytic mammalian cell surface receptor.

[0014] Disclosed is a method for delivering a functional nucleic acid to a target mammalian cell. The inventive methodology comprises (a) providing a plurality of intact minicells in a pharmaceutically acceptable carrier, each minicell of the plurality encompassing plasmid-free functional nucleic acid, and (b) bringing minicells of the plurality into contact with mammalian cells such that the mammalian cells engulf minicells of the plurality, whereby the functional nucleic acid is released into the cytoplasm of the target cells. As mentioned, the functional nucleic acid, exemplified by regulatory RNA such as siRNA, miRNA and shRNA, can target RNA transcripts encoding a protein that contributes to drug resistance, apoptosis resistance or neoplasticity. In other embodiments, the methodology of the invention further comprises delivering a drug, distinct from the functional nucleic acid, to the target mammalian cell. The drug can be administered after or concurrently or even before the administration of the minicell composition.

[0014a] In another aspect, there is provided an in vitro method of delivering functional nucleic acid to a target mammalian cell, comprising: (a) providing a plurality of intact minicells in a pharmaceutically acceptable carrier, each minicell of the plurality comprising plasmid-free functional nucleic acid, and (b) contacting minicells of the plurality with the target mammalian cells such that the mammalian cells engulf minicells of the plurality, whereby the functional nucleic acid is released into the cytoplasm of the mammalian cells.

[0015] Disclosed is a method for formulating a minicell with a plasmid-free functional nucleic acid. The method comprises co-incubating a plurality of minicells with a functional nucleic acid, such as regulatory RNA like siRNA, miRNA or shRNA, in a buffer. In some embodiments, the co-incubation may involve gentle shaking, while in others the co-incubation is static. In some aspects, the co-

incubation lasts about half an hour, while in others it lasts about an hour. In one embodiment, the buffer comprises buffered saline, for example, a 1X phosphate buffer solution. In another embodiment, the co-incubation is conducted at a temperature of about 4°C to about 37°C, about 20°C to about 30°C, about 25°C, or about 37°C. The co-incubation can comprise about 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12} or 10^{13} minicells.

[0015a] In another aspect, there is provided a method for formulating a minicell of the composition as described herein, comprising co-incubating a plurality of intact minicells with functional nucleic acid in a buffer.

[0016] Other objects, features and advantages will become apparent from the following detailed description. The detailed description and specific examples are given for illustration only since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description. Further, the examples demonstrate the principle of the invention and cannot be expected to specifically illustrate the application of this invention to all the examples where it will be obviously useful to those skilled in the prior art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 depicts intact minicells packaged with Cy3 fluorophore-labeled siRNA. Figure 1A is from a light microscope, while Figure 1B shows the same slide but viewed under fluorescent light with a 515-560 excitation filter revealing strongly fluorescent siRNA molecules coincident with the minicells.

[0018] Figure 2 is an image captured by fluorescence confocal microscopy and shows the adhesion and internalization of EGFR-targeted, siRNA-Plk1-packaged minicells into human breast cancer cells *in-vitro*.

[0019] Figure 3 graphically shows a significant anti-tumor effect achieved by treating human breast cancer (MDA-MB-468) xenografts in nude mice with EGFR-targeted, KSP-siRNA-packaged minicells. Control group 1(—◆—) received sterile saline, while experimental group 2 (—⊖—) received ^{EGFR}minicells$_{siRNA\text{-}KSP}$.(10^9), four times per week.

[0020] Figure 4 graphically depicts a significant anti-tumor effect achieved by treating human colon cancer (HCT116) xenografts in nude mice with EGFR-targeted, KSP-siRNA-packaged minicells in conjunction with EGFR-targeted, carboplatin-packaged minicells. Group 1 (—◆—) mice received sterile saline, and mice of groups 2 (-△--), 3 (—■—) and 4

WE CLAIM:

1. A composition comprising:

(a) a plurality of intact minicells, each minicell of the plurality comprising plasmid-free functional nucleic acid, and

(b) at least one pharmaceutically acceptable carrier, wherein the plurality comprises a therapeutically effective amount of the functional nucleic acid.

2. The composition of claim 1, wherein the functional nucleic acid is a DNA or a regulatory RNA.

3. The composition of claim 2, wherein:

(a) the DNA is single-, double-, or multi-stranded DNA;

(b) the DNA is antisense oligonucleotide; or

(c) the regulatory RNA is selected from the group consisting of siRNA, miRNA, shRNA, antisense ssRNA, ribozyme, and decoy RNA.

4. The composition of any one of claims 1-3, further comprising a drug.

5. The composition of claim 4, wherein:

(a) the functional nucleic acid or antisense oligonucleotide targets a transcript encoding a protein that contributes to resistance to the drug; and/or

(b) the drug is packaged in an intact minicell; and/or

(c) the functional nucleic acid and the drug are packaged within the same intact minicell or packaged within different intact minicells.

6. The composition of any one of claims 1-5, wherein:

(a) the functional nucleic acid or antisense oligonucleotide targets an RNA transcript encoding a protein that contributes to apoptosis resistance; or

(b) the functional nucleic acid or antisense oligonucleotide targets an RNA transcript encoding a protein that contributes to neoplasticity; or

(c) the antisense oligonucleotide or antisense oligonucleotide targets the RNA transcript encoding a protein that contributes to drug resistance; or

(d) the functional nucleic acid or antisense oligonucleotide targets a transcript of P-glycoprotein, MDR-2, or MDR-3; or

(e) the functional nucleic acid or antisense oligonucleotide targets a transcript of MRP2, BCR-ABL, STI-571 resistance-associated protein, lung resistance-related protein, cyclooxygenase-2, nuclear factor kappa, XRCC1, ERCC1, GSTP1, mutant β-tubulin, or a growth factor; or

(f) the functional nucleic acid or antisense oligonucleotide targets a transcript of Bcl-2, Bcl-X_L, A1/Bfl 1, focal adhesion kinase or p53 protein; or

(g) the functional nucleic acid or antisense oligonucleotide targets a transcript of β-Catenin, PKC-α, C-RAF, K-Ras, DP97 Dead box RNA helicase, cdk1, DNMT1, FLIP, C-Sfc, 53BPI, Polycomb group protein EZH2, ErbB1, HPV-16 E5 and E7, Fortilin & MCI1P, KSP, DIP13α, MBD2, p21, KLF4, tpt/TCTP, SPK1 & SPK2, P300, PLK1, Trp53, Ras, ErbB1, VEGF, or BAG-1.

7. The composition of any one of claims 1-6, wherein the minicells further comprise a bispecific ligand.

8. The composition of claim 7, wherein the bispecific ligand comprises an antibody or an antibody fragment.

9. The composition of claim 7 or 8, wherein the bispecific ligand comprises: (a) a first arm that carries specificity for a minicell surface structure, wherein the minicell surface structure is an O-polysaccharide component of a lipopolysaccharide on the minicell surface; and (b) a second arm that carries specificity for a non-phagocytic mammalian cell surface receptor, wherein the mammalian cell surface receptor is capable of activating receptor-mediated endocytosis of the minicell.

10. The composition of any one of claims 1-9, wherein the composition comprises:

(a) fewer than about 1 contaminating parent bacterial cell per 10^7 minicells; or

(b) fewer than about 1 contaminating parent bacterial cell per 10^8 minicells; or

(c) fewer than about 1 contaminating parent bacterial cell per 10^9 minicells; or

(d) fewer than about 1 contaminating parent bacterial cell per 10^{10} minicells; or

(e) fewer than about 1 contaminating parent bacterial cell per 10^{11} minicells.

11. The composition of any one of claims 1-10, wherein there is an absence from the minicells of a construct for *in situ* expression of the functional nucleic acid.

12. An in vitro method of delivering functional nucleic acid to a target mammalian cell, comprising:
(a) providing a plurality of intact minicells in a pharmaceutically acceptable carrier, each minicell of the plurality comprising plasmid-free functional nucleic acid, and
(b) contacting minicells of the plurality with the target mammalian cells such that the mammalian cells engulf minicells of the plurality, whereby the functional nucleic acid is released into the cytoplasm of the mammalian cells.

13. The method of claim 12, wherein the functional nucleic acid is a DNA or a regulatory RNA.

14. The method of claim 13, wherein:
(a) the DNA is antisense oligonucleotide; or
(b) the regulatory RNA is selected from the group consisting of siRNA, miRNA, shRNA, antisense ssRNA, ribozyme, and decoy RNA.

15. The method of any one of claims 12-14, further comprising delivering a drug to the target mammalian cell, and wherein optionally the functional nucleic acid or antisense oligonucleotide targets the transcript of a protein that contributes to resistance to the drug.

16. The method of any one of claims 12-15, wherein:
(a) the functional nucleic acid or antisense oligonucleotide targets an RNA transcript encoding a protein that contributes to apoptosis resistance; or
(b) the functional nucleic acid or antisense oligonucleotide targets an RNA transcript encoding a protein that contributes to neoplasticity; or
(c) the functional nucleic acid or antisense oligonucleotide targets the RNA transcript encoding a protein that contributes to drug-resistance; or
(d) the functional nucleic acid or antisense oligonucleotide targets a transcript from P-glycoprotein, MDR-2 or MDR-3; or
(e) the functional nucleic acid or antisense oligonucleotide targets a transcript from MRP2, BCR-ABL, STI-571 resistance-associated protein, lung resistance-related protein,

cyclooxygenase-2, nuclear factor kappa, XRCC1, ERCC1, GSTP1, mutant β-tubulin, or a growth factor; or

(f) the functional nucleic acid or antisense oligonucleotide targets a transcript of Bcl-2, Bcl-X_L, A1/Bfl 1, focal adhesion kinase or p53 protein; or

(g) the functional nucleic acid or antisense oligonucleotide targets a transcript of β-Catenin, PKC-α, C-RAF, K-Ras, DP97 Dead box RNA helicase, cdk1, DNMT1, FLIP, C-Sfc, 53BPI, Polycomb group protein EZH2, ErbB1, HPV-16 E5 and E7, Fortilin & MCI1P, DIP13α, KSP, MBD2, p21, KLF4, tpt/TCTP, SPK1 & SPK2, P300, PLK1, Trp53, Ras, ErbB1, VEGF, or BAG-1.

17. The method of any one of claims 12-16, wherein step (b) comprises contacting a bispecific ligand with at least some of the intact minicells and the target mammalian cell, such that the bispecific ligand causes minicells to bind to the mammalian cell.

18. The method of claim 17, wherein the bispecific ligand comprises: (a) a first arm that carries specificity for a minicell surface structure, wherein the minicell surface structure is an O-polysaccharide component of a lipopolysaccharide on the minicell surface; and (b) a second arm that carries specificity for a non-phagocytic mammalian cell surface receptor.

19. The method of claim 17 or 18, wherein:

(a) the bispecific ligand comprises an antibody or antibody fragment; and/or

(b) the mammalian cell is phagocytosis- or endocytosis-competent; and/or

(c) the mammalian target cell is a non-phagocytic cell.

20. A method for formulating a minicell of the composition of any one of claims 1-11, comprising co-incubating a plurality of intact minicells with functional nucleic acid in a buffer.

21. The method of claim 20, wherein the functional nucleic acid is a DNA or a regulatory RNA.

22. The method of claim 21, wherein:

(a) the DNA is single-, double-, or multi-stranded DNA;

(b) the DNA is antisense oligonucleotide; or

(C) the regulatory RNA is selected from the group consisting of siRNA, miRNA, shRNA, antisense ssRNA, ribozyme, and decoy RNA.

23. The method of any one of claims 20-22, wherein the co-incubation comprises gentle shaking.

24. The method of any one of claims 20-23, wherein the co-incubation lasts about 0.5 hour or about 1 hour.

25. The method of any one of claims 20-24, wherein the buffer comprises: (a) buffered saline; (b) buffered saline in gelatin form; or (c) a 1X phosphate buffer solution.

26. The method of any one of claims 20-25, wherein the co-incubation is conducted at a temperature selected from the group consisting of:

(a) about 4°C to about 37°C;

(b) about 20°C to about 30°C;

(c) about 25°C; or

(d) about 37°C.

27. The method of any one of claims 20-26, wherein the co-incubation comprises about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12} or about 10^{13} minicells.