SUMMARY ~~OF THE INVENTION~~

[0011a] In one aspect, there is provided a composition comprising (a) a plurality of intact, bacterially-derived minicells, wherein each minicell of said plurality encompasses regulatory RNA that is packaged in said minicell, and (b) a pharmaceutically acceptable carrier therefor, wherein there is an absence from said minicells of a construct for in situ expression of said regulatory RNA, wherein said regulatory RNA is selected from the group consisting of siRNA, miRNA and shRNA, and wherein said plurality contains a therapeutically effective amount of said regulatory RNA.

[0012] In accordance with one ~~aspect~~embodiment of the ~~invention~~disclosure, therefore, 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.

[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~~disclosure 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] In another ~~aspect~~embodiment of the ~~invention~~disclosure, a method is provided 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~~disclosure 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 one aspect, there is provided the composition as defined above for use as a medicament.

[0014b] In one aspect, there is provided the composition as defined above for use in the treatment of drug-resistance or apoptosis resistance.

[0014c] In one aspect, there is provided the composition as defined above for use in the treatment of cancer or neoplasticity, further comprising a bispecific ligand, said bispecific ligand comprising a first arm that carries specificity for a minicell surface structure and a second arm that carries specificity for a non-phagocytic mammalian cell surface receptor.

[0014d] In one aspect, there is provided a method for formulating minicells of the composition as defined above, comprising co-incubating in a buffer (A) a plurality of intact, bacterially-derived minicells with (B) plasmid-free, regulatory RNA, thereby obtaining minicells that contain said regulatory RNA, wherein said regulatory RNA is selected from the group consisting of siRNA, miRNA and shRNA.

[0015] In accordance with another ~~aspect~~embodiment, the present ~~invention~~disclosure contemplates 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~~embodiments, 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.

[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~~disclosure will become apparent to those skilled in the art from this detailed description. Further, the examples demonstrate the principle of the ~~invention~~disclosure and cannot be expected to specifically illustrate the application of this ~~invention~~disclosure 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 $^{EGFR}\text{minicells}_{\text{siRNA-KSP}}.(10^9)$, 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