Affinity-Targeted Silver Nanoparticles as a Research Tool and a Drug Carrier

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ABSTRACT

We have developed silver nanoparticles (AgNPs) as a model carrier system to study tumor homing peptide-mediated targeting and cellular/tissue interactions of AgNPs *in vitro* and *in vivo*. AgNPs loaded with fluorescent dyes can be tracked in cells and tissues by ultrasensitive optical imaging, as the silver cores of the AgNPs plasmonically enhance the fluorescent signal [1]. To allow quantitative internally-controlled cellular uptake and tissue biodistribution studies using AgNPs, we have developed isotopically barcoded AgNPs and optimized their detection by inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation ICP-MS. This approach allows ultrasensitive parallel auditioning of peptide-guided and control AgNPs in the same biological test systems [2,3]. Furthermore, peptide-targeted AgNPs can serve as a carrier for cytotoxic payloads to receptor-positive cancer cells [4].
In addition to the synthesis and characterization of the nanoparticles, we show that AgNPs loaded with a potent anticancer drug, monomethyl auristatin E (MMAE), and targeted with Neuropilin-1-targeting C-end Rule peptide, RPARPAR, accumulate in prostate cancer cells overexpressing the receptor protein and cause selective toxicity in these cells through the release of MMAE by lysosomal cathepsin B (Figure 1). Importantly, the specific cytotoxic activity of RPARPAR-MMAE-AgNPs (vs. non-targeted MMAE-AgNPs) can be potentiated by dissolution of the extracellular nanoparticles with a mild biocompatible etching solution [1]. We demonstrated that the binding and subsequent internalization is Neuropilin-1-dependent (Figure 2), and quantified the uptake of MMAE, which was delivered to cells on AgNPs, by HPLC-MS. RPARPAR-MMAE-AgNPs were also tested in a mixed culture of target and control cells, where specific elimination of target cancer cells was clearly evident. Several assays were used to evaluate the cytotoxicity of these nanoparticles.
These studies suggest that the AgNP platform can be used for quantitative nanoparticle biodistribution studies and as an anticancer drug carrier *in vitro*, and that elimination of extracellular AgNPs by exposure to etching solution can be used as an endocytosis research tool and as a means to improve the therapeutic index of AgNPs loaded with anticancer payloads. Validation of peptide-targeted MMAE-AgNPs as an effective drug carrier *in vivo* are currently under way.

References

[1] G. B. Braun, T. Friman, H.B. Pang, A. Pallaoro, T. H. De Mendoza, A.M. A. Willmore, V. R. Kotamraju, A. P. Mann, Z. G. She, K. N. Sugahara and N.O. Reich, Nature Materials, 13, 9 (2014) 904.
[2] A. M. A. Willmore, L. Simon-Gracia, K. Toome, P. Paiste, V. R. Kotamraju, T. Molder, K. N. Sugahara, E. Ruoslahti, G. B. Braun and T. Teesalu, Nanoscale, 8, 17 (2016) 9096-9101.
[3] K. Toome, Kadri, A. M. A. Willmore, P. Paiste, A. Tobi, K. N. Sugahara, K. Kirsimäe, E. Ruoslahti, G. B. Braun and T. Teesalu. Nanoscale, 9, 28 (2017), 10094-10100.
[4] H. B. Pang, G. B. Braun, T. Friman, P. Aza-Blanc, M. E. Ruidiaz, K. N. Sugahara, T. Teesalu and E. Ruoslahti, Nature communications, 5 (2014), 4904.

Figures

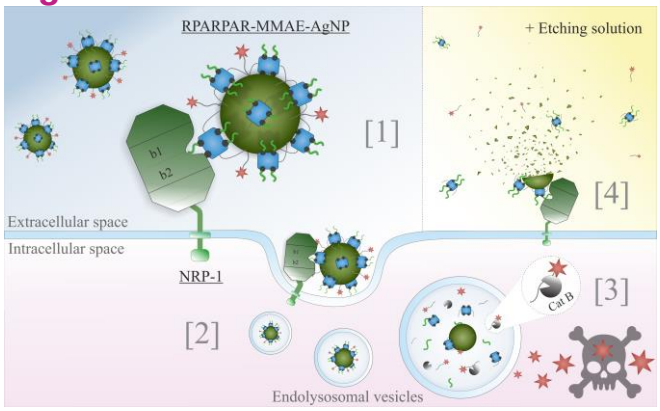

Figure 1. CendR peptide guided conditional therapeutic AgNPs. [1] RPARPAR-MMAE-AgNPs bind to the b1b2 binding pocket of NRP-1 on the surface of cells. [2] Binding to NRP-1 initiates cellular uptake of particles in the endolysosomal compartment. [3] Intracellular RPARPAR-MMAE-AgNPs are processed by cathepsin B (Cat B) at Val-Cit bond on the linker incorporated into the cytotoxic MMAE (red stars) to release the active MMAE in the cytosol where it binds to tubulin and prevents cell division, eventually causing cell death. [4] The extracellular fraction of the AgNPs can be removed by a mild biocompatible etching solution consisting of hexacyanoferrate and thiosulfate.

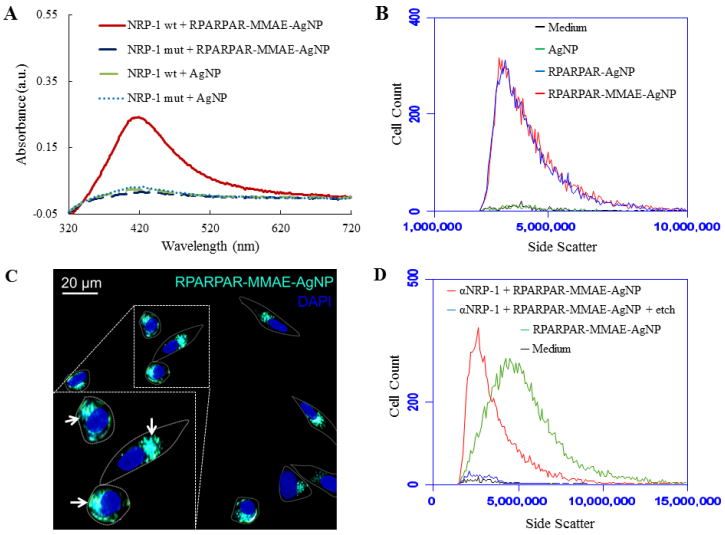

Figure 2. Neuropilin-1-dependent binding and internalization of RPARPAR-MMAE-AgNPs. (A) RPARPAR-MMAE-AgNPs bind to the b1b2 domain of NRP-1. Recombinant wild type (wt) and mutant (mut) b1b2 domains of Neuropilin-1 (NRP-1) with a 6xHis-tag were coupled to Ni-NTA magnetic agarose beads. After 1 h incubation with AgNPs (1.5 nM) at 37 °C the unbound AgNPs were removed by washing, bound AgNPs in complex with NRP-1 were released from the beads with imidazole, and absorbance of the eluate in the UV-Vis range was measured. (B) RPARPAR functionalization renders AgNPs and MMAE-AgNPs selective for NRP-1 binding. Cells in suspension were incubated with 1.5 nM AgNPs at 37 °C for 1 h, washed, and resuspended in PBS for flow cytometry to measure AgNP uptake in the cells. Representative data are shown (n = 3). See Fig. S1 for data on the NRP-1 negative control cell line. (C) Representative dark-field image (with DAPI overlay) showing internalization of RPARPAR-MMAE-AgNPs in PPC-1 cells. Attached PPC-1 cells were incubated with 1.5 nM RPARPAR-MMAE-AgNPs (green) at 37 °C for 1 h. Extracellular AgNPs were removed by exposure to 10 mM etchant for 5 min. The cells were fixed with –20 °C MeOH, counterstained with DAPI (1 µg/ml; blue), and imaged using an inverted microscope. The image is a composite of dark-field and DAPI images; dotted lines outline the cells, arrows point to internalized AgNPs; scale bar: 20 µm. (D) NRP-1-dependent internalization of RPARPAR-MMAE-AgNPs by PPC-1 cells. PPC-1 cells in suspension were incubated with 1.5 nM AgNPs at 37 °C for 1 h, washed, and analysed by flow cytometry. Optional incubation with etchant prior to flow cytometry for 5 min was used to remove extracellular surface-bound AgNPs. To study the role of the NRP-1 in the cellular interaction of particles, the cells were incubated with 10 µg/mL function-blocking polyclonal rabbit α-NRP-1 antibody at 37 °C for 15 min prior to incubation with AgNPs. Representative data are shown (n = 3).