Silver nanoparticles selectively treat neurofibromatosis type 1-associated malignant peripheral nerve sheath tumors

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Abstract

Objectives: Neurofibromatosis type 1 (NF1) is an inherited disorder characterized by loss-of-function mutations in the neurofibromin gene. NF1 patients are susceptible to development of neurofibromas which can transform into deadly malignant peripheral nerve sheath tumors (MPNSTs) after complete loss of neurofibromin. NF1-associated MPNSTs have a generally poor prognosis. Our group and others show that rationally developed nanomedicines show promise in cancer therapy. Here we evaluate silver nanoparticles (AgNPs) as a cancer-selective therapy for NF1-associated MPNSTs to address a significant unmet clinical need.

Methods: A panel of in vitro cell models of NF1-associated MPNSTs, sporadic MPNSTs, and normal Schwann cells were employed to evaluate cancer-selectivity of AgNP relative to standard of care. We then used miRNA or neurofibromin expression vectors to either decrease or restore functional neurofibromin expression, respectively.

Results: Our data show that AgNPs are selectively cytotoxic to NF1-associated MPNSTs relative to sporadic MPNST and normal Schwann cells. Sensitivity to AgNP was potentiated when co-treated with oxidative stress inducing agents. Furthermore, we found that sensitivity to AgNPs is correlated with expression levels of functional neurofibromin. Restoration of neurofibromin expression in NF1-associated MPNSTs decreased AgNP sensitivity and reducing neurofibromin expression in Schwann cells increased AgNP sensitivity. This finding is unique to AgNP as our alterations in neurofibromin expression did not alter susceptibility to doxorubicin. Using a co-culture model system, we found that AgNP selectively eradicated NF1-associated MPNST at doses allowing normal Schwann cells to remain viable.

Conclusions: We show that NF1-associated MPNSTs are susceptible to AgNP at doses that are tolerated by healthy Schwann cells. These data support the use of AgNP as a novel approach for clinical management of NF1-associated MPNSTs and warrants further preclinical evaluation.

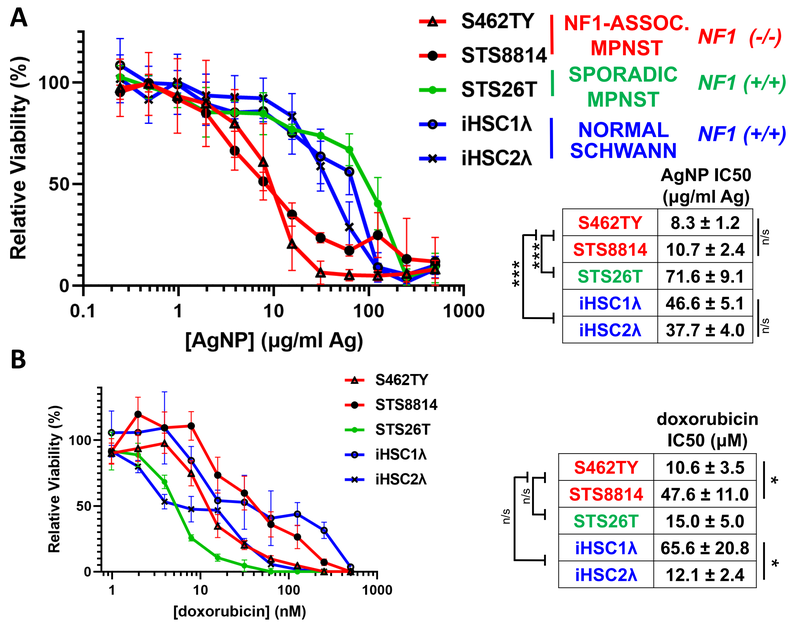

Figure 1: **AgNPs show selective cytotoxicity in NF1-null MPNSTs relative to *NF1-wildtype* MPNSTs and normal Schwann cells whereas standard of care doxorubicin does not show selectivity. (A)** NF1-associated *NF1-null* MPNSTs (S462TY, STS8814), sporadic *NF1-wildtype* MPNST (STS26T), and immortalized Schwann cells (iHSC1λ, iHSC2λ) were exposed to **(A)** AgNPs (25 nm, polyvinylpyrrolidone coated, nanoComposix) of **(B)** standard of care doxorubicin for 72 hours and viability assessed by MTT assay. Cell model identification is displayed and IC50 of AgNP and doxorubicin is shown by μg/ml Ag or μM doxorubicin ± SEM. Significance between and within groups was determined by one-way ANOVA and student's t-test where appropriate (*** p<0.005, * p<0.05, n/s not significant).

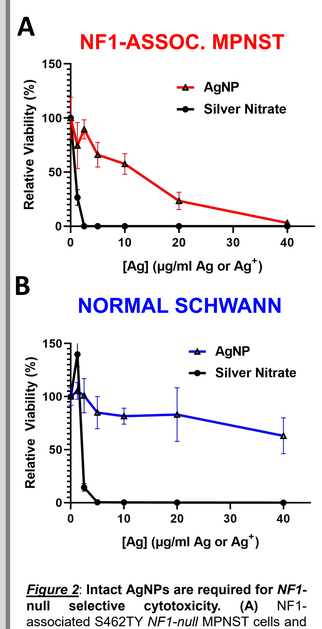

Figure 2: **Intact AgNPs are required for *NF1*-null selective cytotoxicity. (A)** NF1-associated S462TY *NF1-null* MPNST cells and **(B)** normal Schwann cells (iHSC1λ) were exposed to AgNPs (intact silver nanoparticles) or AgNO3 (silver ion -Ag^+) at equivalent Ag doses for 48 h and viability assessed by MTT. Data is shown ± SD and is representative of three independent experiments.

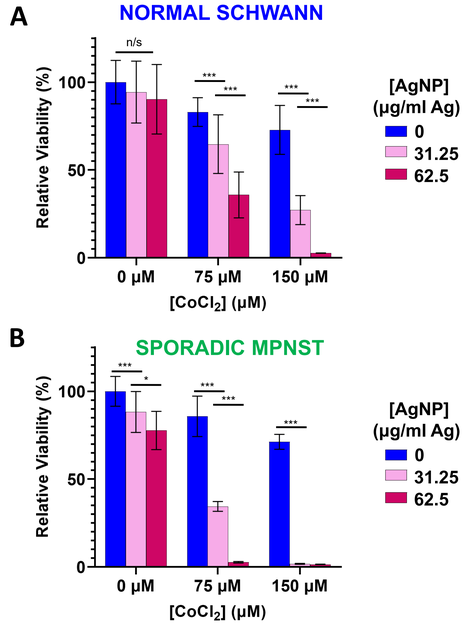

Figure 3: **Cobalt chloride-induced hypoxic/oxidative stress sensitizes normal Schwann cells and sporadic *NF1*-wildtype MPNST to AgNP treatment. (A)** normal Schwann cells (iHSC1λ) or **(B)** sporadic NF1-wildtype MPNSTs (STS26T) were exposed to $CoCl_2$ and AgNPs for 48 h and viability assessed by MTT. Data is shown ± SD and is representative of three independent experiments. Significance between treatments was determined by Student's t-test (*** p<0.005, * p<0.05, n/s not significant).

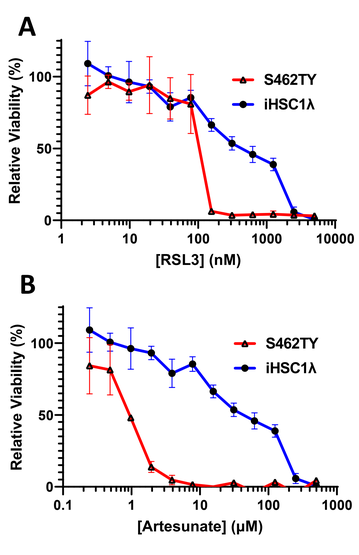

Figure 4: **Neurofibromatosis type 1-associated malignant peripheral nerve sheath tumors are more sensitive to ferroptosis inducing agents compared to normal Schwann cells.** NF1-associated S462TY *NF1-null* MPNST cells and normal Schwann cells (iHSC1λ) were treated with ferroptosis inducing agents **(A)** RSL3 (0 – 5,000 nM) or **(B)** artesunate (0 – 500 μM) for 72 h and viability assessed by MTT. Data is shown ± SD and is representative of three independent experiments each containing five technical replicates.

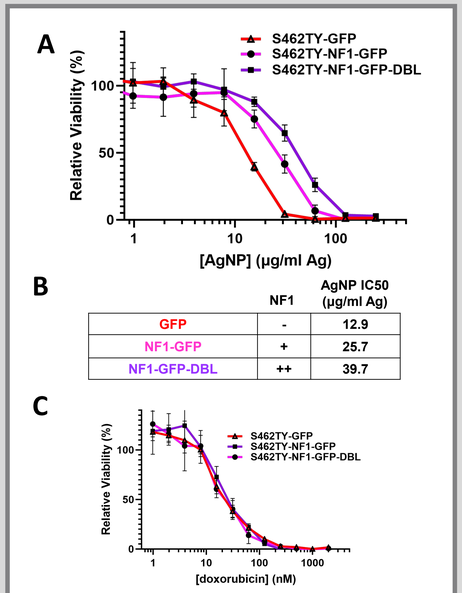

Figure 5: **Restoration of *NF1* in NF1-associated MPNST reduces sensitivity to AgNPs with no change in sensitivity to standard of care doxorubicin.** NF1-associated *NF1-null* MPNST cell line S462TY was transfected with expression plasmids contacting control GFP or Neurofibromin 1 –tagged with GFP and puromycin selected. **(A)** Cells were then treated with AgNP (0-250μg/ml Ag) for 72 hours and viability assessed by MTT assay. **(B)** IC50 of AgNP is shown by μg/ml Ag. **(C)** Cells were treated with doxorubicin (0-2000 nM) for 72 hours and viability assessed by MTT assay. Data is representative of four independent experiments each containing five technical replicates.

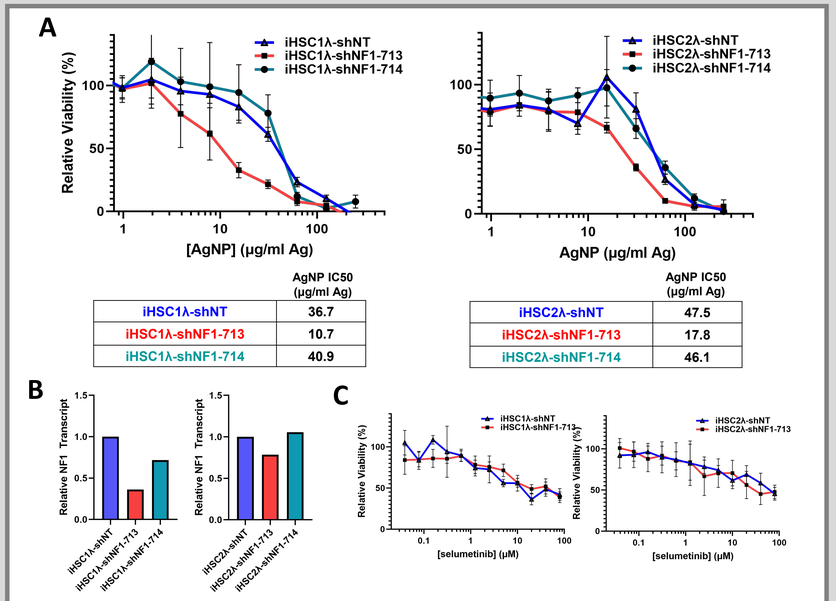

Figure 6: **Knockdown of *NF1* increases sensitivity to AgNPs in normal Schwann cells with no change in sensitivity to standard of care selumetinib.** Normal Schwann cell lines iHSCλ1 and iHSCλ2 were transfected with non-targeting control shNT or shNF1 (713 or 714) and puromycin selected. **(A)** iHSCλ1-shNT, -shNF1-713, and -shNF1-714 (*left*) or iHSCλ2-shNT, -shNF1-713, and -shNF1-714 (*right*) were treated with AgNP (0-500 μg/ml Ag) for 72 hours and viability assessed by MTT assay. IC50 of AgNP is shown as μg/ml Ag. **(B)** qPCR specific for *NF1* and housekeeping gene *PPIA* was performed in quadruplicate and relative *NF1* transcript levels calculated using ΔΔCT methods are shown. **(C)**. iHSCλ1-shNT and -shNF1-713 (*left*) or iHSCλ2-shNT, -shNF1-713 (*right*) were treated with standard of care selumetinib for 72 hours and viability assessed by MTT. Data is representative of three independent experiments each containing five technical replicates

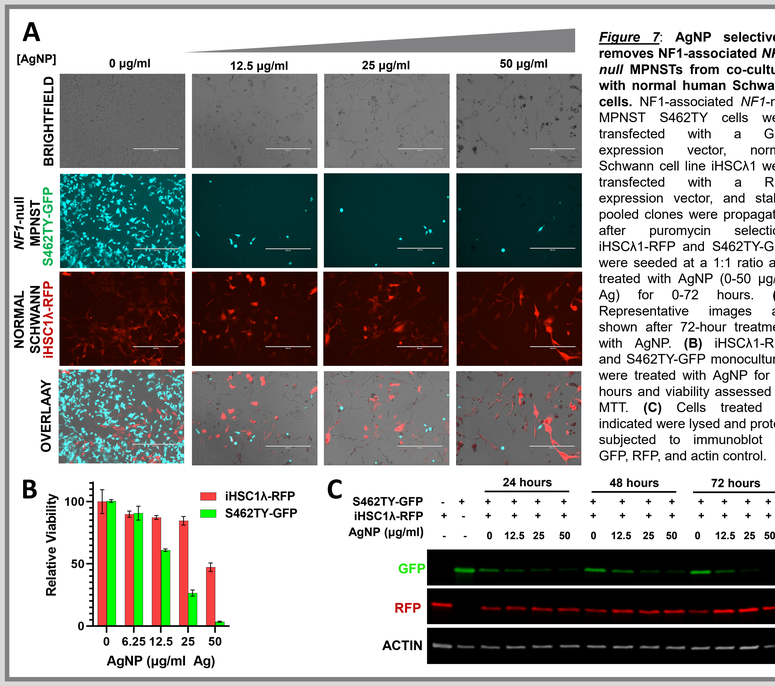

Figure 7: **AgNP selectively removes NF1-associated *NF1-null* MPNSTs from co-culture with normal human Schwann cells.** NF1-associated *NF1*-null MPNST S462TY cells were transfected with a GFP expression vector, normal Schwann cell line iHSCλ1 were transfected with a RFP expression vector, and stable pooled clones were propagated after puromycin selection. iHSCλ1-RFP and S462TY-GFP were seeded at a 1:1 ratio and treated with AgNP (0-50 μg/ml Ag) for 0-72 hours. **(A)** Representative images are shown after 72-hour treatment with AgNP. **(B)** iHSCλ1-RFP and S462TY-GFP monocultures were treated with AgNP for 72 hours and viability assessed by MTT. **(C)** Cells treated as indicated were lysed and protein subjected to immunoblot for GFP, RFP, and actin control.

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