Electronic Supplementary Material (ESI) for Nanoscale.
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Materials and Methods

Mice and Eμ-myc-derived cell line establishment.

Eμ-myc transgenic mice (TgN(IghMyc)22Bri/J) were obtained from The Jackson Laboratory (Bar Harbor, Maine; USA). The Bioethical Committee of the University Magna Graecia of Catanzaro approved the experimental protocols. Animal experiments were carried out in accordance with the protocol n.794/2016-PR approved by the Italian Ministry of Health. Mice were monitored daily for signs of morbidity and tumor development. Three weeks later, the mice were sacrificed via cervical dislocation, lymph nodes were trypsinized and cultivated for 3 days in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. Following the same procedures, stable populations of (1×10^6) Eμ-myc-derived cells were established and re-inoculated. Following four rounds of selection from the parental cells, a stable Eμ-myc-derived cell line was selected at random and used in the following experiments.

In vivo phage display screening.

Ph.D.-C7C Phage Display Peptide Library kit was purchased from New England Biolabs, Ipswich, MA, US. Panning was performed injecting 1×10^{11} virions directly in the tail vain of tumor harboring mice. After 1 hour of circulation, the mice were perfused to remove unspecific binders. Target organs were collected, phages from tumor mass were amplified and used for the following cycles. After three *biopanning* cycles, twenty independent phages were randomly chosen to retrieve the selected epitopes' amino acid sequence

Phage labelling with FITC

Labeling of phages was carried out employing fluorescein isothiocyanate (FITC) as fluorophore. An aliquot of $1x10^{13}$ pfu/μL of phages was precipitated with standard

polyethylene glycol 8000 (PEG 8000; Sigma) and NaCl solution and subsequently re-suspended in 200 μL of conjugation buffer in microfuge tubes. The phage/fluorochrome (10 μL of FITC solution) reaction was allowed to continue for 1 h at room temperature in the dark. After incubation, the phages were precipitated twice with 200 μL of PEG and NaCl. Additional rounds of PEG precipitation minimized residual free FITC, and the final pellet was solubilized in 200 μL of PBS buffer.

ex vivo binding validation

Cell suspensions from tumor masses of ten Eμ-myc tumor-bearing mice were obtained by grinding and filtering tissues through 0.4 μm cell strainers (BD Biosciences) in PBS. The suspension was transferred to a fresh tube for centrifugation at 1,000 × g for 5 min. The cell pellet from spleen was resuspended in red blood cell lysis buffer (Sigma), incubated for 1 min at room temperature, resuspended in PBS, and centrifuged for 1,000 × g for 5 min. Cell pellet was incubated with fluorescent-conjugated phages (dilution 1:100 in PBS) for 15 min at 4°C in the dark, then washed and analyzed by flow cytometry.

Immunohistochemistry

Tissue specimens were fixed with 4% PFA, embedded in Optimal Cutting Temperature Compound (OCT) and cut in 10 □m cross-sections for immunohistochemistry analysis. To prepare tissue for immunostaining, slides were washed with Phosphate Buffered Saline (PBS). Non-specific antibody binding was blocked by incubation with 10% normal donkey serum (Jackson ImmunoResearch) for 30 minutes at room temperature. The ex vivo

blinders validation was performed through double immunostaining with Wheat Germ Agglutinin (WGA) Alexa Fluor 647 conjugate (1:200 dilution; Invitrogen) and the FITC-conjugated paghes. The antibodies were incubated 30 minutes at room temperature, respectively. The nuclei were counterstained with the DNA binding dye, DAPI (4, 6-diamidino-2-phenylindole, Sigma) at 1 □g/ml and mounted using Vectasheild mounting media (Vector labs). Imagines were acquired using a confocal microscope (LEICA TCS SP5 and SP8).

Imaging *in vivo*

Imaging was performed with the Bruker In-Vivo Xtreme X-ray/optical imaging system, equipped with a highly sensitive cooled charge-coupled camera mounted in a light-tight box (Bruker, In-Vivo Xtreme, Billerica, MA). 120 minutes after tail vein administration of FITC-conjugated phages, animals were anesthetized with 4% isoflurane, in an anesthesia induction chamber, and then placed in the air-tight box in the optical imaging chamber with continuous inflow of 2.5% isoflurane in 2% oxygen. The animal's body temperature was monitored and kept constant at 37 °C, by a ventilation system. Fluorescence images were acquired at an exposure time of 2 sec, with an emission wavelength of 480 nm, an emission wavelength of 535 nm, 15×15 cm field of view (FOV), fractional stop (F/stop) 2; 1×1 binning. X- ray images were acquired at an exposure time of 1.2 sec., 15×15 cm field of view (FOV), fractional stop (F/stop) 2.

Exosomes purification and physical characterization

Exosomes were size exclusion isolated from 500 □L of sera using qEVoriginal/70nm columns (Izon, Izon Science Ltd.) according to the manufacturer's protocol. The size distribution and concentration of EV particles were evaluated with Tunable resistive pulse sensing, TRPS method (qNano, Izon Science Ltd.) by using a NP100 nanopore membrane at with 47,5 mm stretch. All measurements were calibrated by using 110 nm polystyrene calibration beads appropriately diluted (CPC 100, Izon Science Ltd.) in the same measurements condictions. Sample analysis was carried out on the Izon Control Suite software v3.3 (Izon Science, UK).

Exosome immunocapture and flow cytometry

Isolated exosomes from serum were suspended in 1 ml of 1X PBS. Exosome aliquots (500 □l) were labeled with 50 □l of 10X Exo-Red [System Biosciences – SBI] according to manufacturer's instructions. The exosomes were re-isolated using the addition of 100 □l ExoQuick followed by precipitation for 30 minutes at + 4°C. The labeled exosome pellet was suspended in 500 □l 1X PBS and stained with CD63-coupled magnetic beads provided by SBI's Exo-Flow IP kit [SBI] and with the FITC-conjugated phages.

AFM and ESEM analysis.

Environmental Scanning Electron Microscope (ESEM, FEI Quanta FEG 400) was used at 8.0KV and at 10.0KV, flat surface silicon chips were used as a support. Atomic Force Microscope (AFM, Bruker Multimode 8 equipped with a Nanoscope V controller) was

operated in tapping mode employing probes with a resonance frequency of 150kHz (RTESPA-150, Bruker). Samples were deposited on freshly cleaved mica and the imaging was performed in air.

Reverse transcription and qPCR Approximately

20–25 ng RNA isolated from each pool of exosomal samples was reverse transcribed to cDNA by using the miRcury Locked Nucleic Acid (LNA) Universal Reverse Transcription miRNAs PCR, Polyadenylation, and cDNA Synthesis Kit following the manufacturer's protocol (Exiqon; Qiagen, Germantown, MD, USA). We used the Exiqon; Qiagen miRcury-Ready-to-Use-PCR -Panel-I + II V4 (Exiqon; Qiagen) to screen the expression levels of miRNAs in serum. qPCR was carried out in accordance with the specifications of miRNA qPCR SYBR Green Kit (Exiqon; Qiagen) to validate the differentially expressed miRNAs in circulating serum exosomes.