Author Correction: Glypican-1 identifies cancer exosomes and detects early pancreatic cancer

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We regret several errors in our publication "Glypican-1 identifies cancer exosomes and detects early pancreatic cancer" and wish to correct them and provide below the explanations and corrected figures. These corrections do not alter the interpretation or conclusions of the study.

The electron microscopy (EM) photos in the manuscript are correct. Nonetheless, some panels of Fig. 1b and 2c and Extended Data Fig. 1c have an incorrect scale bar due to an error in manually copying the bars from the source file. Please see the corrected figures. The calculations of the extracellular vesicles' diameters using TEM are correct because they were done using the source files.

In Fig. 4d, the calculation of the average percentage of $GPC1^+$ crExos at week 7 used three mice when there were actually six mice in the group. This changes the average percentage of $GPC1^+$ crExos for week 7 from 33.53% to 41.93%. This error made the published results more conservative and understated. In the figure legend, it should state "Student *t* test" instead of ANOVA for panel d. The figure legend for panel d should indicate that the solid red line shows the percentage of $GPC1^+$ crExos for the control group, whereas the dashed red line shows the percentage of $GPC1^+$ crExos for the PKT group. Technical replicates correspond to flow cytometry measurements that were performed three times for each mouse serum sample. The statistical analyses reflect a comparison of the percentage of $GPC1^+$ crExos in the control versus PKT groups at each time point using a sample value (obtained by averaging the technical replicates) for each mouse. In addition, the corrected Pearson correlation value for the correlation of tumour volume and percent $GPC1^+$ crExos is $r = 0.604$, $P = 0.002$ for Fig. 4c, d and Extended Data Fig. 6d. The correct sentence for the legend to Fig. 4d should be "**d**, Tumour volume and percentage of $GPC1^+$ crExos in PKT mice at indicated age (Student *t* test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$)."

In Extended Data Fig. 3d with respect to the concentration of exosomes, some of the values were input incorrectly (10^8 versus 10^9) when the data were transferred from the source data file to the software that generates the graphs (4 of 100 samples in the healthy cohort; 1 of 190 samples in the PDAC cohort), resulting in a very slight variation in the graph. The concentration of exosomes in the breast cancer samples continues to be significantly higher when compared with healthy individuals (Student *t* test or ANOVA). The text should read "The relative concentration of crExos was significantly higher in the sera of patients with breast cancer compared to healthy donors (Extended Data Fig. 3d)." No other figures or panels of the manuscript were affected by this error.

In Extended Data Fig. 6a, we inadvertently used the wrong panel for the disease timeline schematic as the correct panel should have depicted the histology of a PKT mouse (normal healthy histology) without lesions rather than the healthy pancreas from a WT mouse. The disease timeline schematic in Extended Data Fig. 6a should have included the middle panel of Extended Data Fig. 7a and not the left panel. Extended Data Fig. 6a is only a schematic representation of disease and not original data. A revised panel is provided.

Because it is not possible to correct the original Article online, the Supplementary information in this amendment contains the corrected figures.

We would also like to correct mistakes we have identified in the text of the manuscript.

In the sentence "NanoSight nanoparticle tracking analysis and transmission electron microscopy (TEM) showed extracellular vesicles of 105 ± 5 nm (mean ± s.d.) and 112 ± 4 nm in diameter, respectively" the word "mean" should instead read "mode," and "(mean ± s.d.)" should have been stated after "112 ± 4 nm." The figure legend for Extended Data Fig. 1a correctly lists the size mode as 105 nm.

In the Extended Data Fig. 4 legend, there are references to ROC curve analysis depicted in Fig. 1. Fig. 1 does not have any ROC curve analyses. The correct sentences in the figure legend for Extended Data Fig. 4a, g are "**a**, Table associated with ROC curve analysis depicted in Fig. 2f. […] **g**, Table associated with ROC curve analysis depicted in Fig. 2h."

The *n* value listed in the figure legend associated with Extended Data Fig. 5e should be $n = 5$ for BPD, not $n = 6$. The graphical depiction of the actual data is correct (Extended Data Fig. 5e). After $GPC1^+$ exosomes were evaluated in the six patients, there was enough serum left for only five of the six patients for ELISA analysis. The corrected sentence in the Extended Data Fig. 5 legend is "**e**, Scatter plots depicting serum GPC1 (ng ml^{-1}) levels by ELISA in patients with BPD ($n = 5$), PDAC ($n = 56$) and healthy controls ($n = 20$) (ANOVA, post hoc Tukey–Kramer test, $^{****}P < 0.0001$; 3 technical replicates)." The corrected sentences in the text are "Next, we evaluated whether an ELISA for circulating GPC1 could function with the same specificity and sensitivity as $GPC1^+$ crExos. Serum samples of the validation cohort (20 healthy donors, 5 patients with BPD and 56 patients with PDAC) were analysed for circulating GPC1 levels."

The label "Extended Data Fig. 2c" in Supplementary Fig. 1 (uncropped blot) is incorrect and should refer to Extended Data Fig. 3c.

On page 181, the reference to "Extended Data Table 3" should be amended. This table was part of the initial manuscript, but it was merged with another table due to editorial request during preparation of the final version of the paper. The corresponding data are presented in Extended Data Table 1b. In the text, the reference to "Extended Data Table 3" should read "Extended Data Table 1b."

On page 180, we state "In a longitudinal study, we bled PKT and littermate control mice repeatedly at 4, 5, 6, 7 and 8 weeks of age ($n = 7$ PKT mice and $n = 6$ control mice; Fig. 4a). Then 3 out of 7 PKT mice were euthanized by week 7, along with 4 out of 6 controls, while the remaining 3 PKT mice and 2 controls were euthanized at week 8." This text is incorrect. The phrase "3 out of 7 PKT mice" should be removed from the second sentence. At week 7, only 6 PKT mice were still alive, as indicated in the Fig. 4 legend: "Longitudinal blood collection of control and PKT mice, at 4 ($n = 6$ and $n = 7$, respectively), 5 ($n = 6$ and $n = 7$), 6 ($n = 6$ and $n = 6$), 7 ($n = 6$ and $n = 6$)". One of the 7 PKT mice died between week 5 and week 6. The corresponding data presented in the Fig. 4a, b legend are correct. The sentences in the text should read "In a longitudinal study, we bled PKT and littermate control mice repeatedly at 4, 5, 6, 7 and 8 weeks of age ($n = 7$ PKT mice and $n = 6$ control mice;

Fig. 4a). Then, 3 out of the remaining 6 PKT mice were euthanized by week 7, along with 4 out of 6 controls, while the remaining 3 PKT mice and 2 controls were euthanized at week 8."

At the end of the results section, on page 181, the sentence "In 4 out of 7 PKT mice with no observed histological lesions, downstream signals for Kras activation, such as phosphorylated ERK (pERK), were detected in the pancreas tissue (Fig. 4j and Extended Data Fig. 7a)" is incorrect. It should read "In 3 out of 7 PKT mice with no observed histological lesions, downstream signals for Kras activation, such as phosphorylated ERK (pERK), were detected in the pancreas tissue (Fig. 4j and Extended Data Fig. 7a)." The data presented in Extended Data Table 1b are correct.

The average and range for the percentage of $GPC1^+$ crExo beads in healthy donors are 3.16% and 0.22–7.6%, respectively. The sentence on page 178 lists incorrect values of "2.3% and 0.3 to 4.7%." It should instead read "…ranging from 0.22% to 7.6% (average of 3.16%; Fig. 2a)." The original graph in Fig. 2a remains correct (the average and range for the percent $GPC1^+$ crExo beads are 3.16% and 0.22–7.6%). ROC and statistical analyses are also correct as reported.

The statistical analysis in Fig. 2a, e and Extended Data Fig. 2b and 3d are correct. The graphical representation with the corresponding significance is also correct. However, the post hoc ANOVA test is the Tukey–Kramer test. In the figure legends, this test was inadvertently described as the Tamhane T_2 test.

We would like to add corrections and extend our Methods section.

The Methods section mentions KPC mice. These mice were not part of the final published manuscript and the manuscript only contains data pertaining to PKT mice. The section describing the mass spectrometry analysis in the original paper is incorrect. We performed two different mass spectrometry analyses: amino acid analysis (not included in this paper) and protein analysis. We inadvertently included the methods for amino acid analysis instead of the protein analysis. We regret the confusion that led to this error. Therefore, the methods for UPLC–MS in the manuscript should be replaced by the methods for nano-liquid chromatography mass spectrometry (LC-ESI-MSM), which can be found in full in the *Corrected Methods* section associated with this amendment. In accordance with this correction, references to "ultra-performance liquid chromatography–mass spectrometry (UPLC–MS)" throughout the text instead refer to the use of "nano-liquid chromatography mass spectrometry (LC-ESI MSM)." Finally, we inadvertently omitted the details of the primers used for mouse *Kras*-associated qRT–PCR experiments. These are listed in the *Corrected Methods* below.

We also provide additional information regarding the methods and antibodies used in our study in the section titled "Flow cytometry sorting of exosome-bound beads," and more data are available at https://www.biorxiv.org/content/10.1101/145706v3.

Corrected Methods

Liquid chromatography and mass spectrometer analysis

Exosomal proteins were extracted using 8 M urea/2.5% SDS. Total protein concentration was determined using micro-BCA protein assay (Pierce). For digestion, 20 µg of each exosome protein isolate was precipitated by methanol/chloroform method. Protein pellets were resuspended and denatured in 20 µl of 50 mM triethylammonium bicarbonate (TEAB)/50% trifluoroethanol (TFE, Sigma-Aldrich), reduced with 10 mM Tris (2-carboxyethyl) phosphine (TCEP, AB SCIEX), pH 8.0, at 60 °C for 60 min and followed by 2 µl cysteine-blocking reagent (methyl methanethiosulfonate (MMTS, Pierce) for 10 min at room temperature. Samples were diluted up to 200 µl, to dilute TFE concentration with 50 mM TEAB. Digestions were initiated by adding 2 µl (1 µg μl^{-1}) sequence-grade modified trypsin (Sigma-Aldrich) to each sample in a 1/10 (wt/wt) ratio, which were then incubated at 37 °C overnight on a shaker. Sample digestions were stopped with 1 µl of 1% trifluoroacetic acid and evaporated to dryness in a vacuum concentrator. The resulting peptide mixture was desalted using a PepClean C18 Spin column (Pierce), following the manufacturer's indications; cleaned tryptic peptides from either in-solution digestion were evaporated to dryness and stored at −20 °C for further analysis. A 2-µg aliquot of each sample was subjected to 2D nano LC-ESI-MS/MS analysis using a nano liquid chromatography system (Eksigent Technologies nanoLC Ultra 1D plus, AB SCIEX) coupled to a high-speed Triple TOF 5600 mass spectrometer (AB SCIEX) with a duo spray ionization source. The analytical column used was a silica-based reversed-phase C18 ChromXP column (75 µm × 15 cm, 3 µm particle size and 120 Å pore size; Eksigent Technologies, AB SCIEX). The trap column was a C18 ChromXP (Eksigent Technologies, AB SCIEX; 3 µm particle diameter, 120 Å pore size) switched online with the analytical column. The loading pump delivered a solution of 0.1% formic acid in water at 2 µl min^{-1}. The nano-pump provided a flow rate of 300 nl min^{-1} and was operated under gradient elution conditions, using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. Gradient elution was performed according to the following scheme: isocratic conditions of 98% A:2% B for 1 min, a linear increase to 30% B in 110 min, a linear increase to 40% B in 10 min, a linear increase to 90% B in 5 min, isocratic conditions of 90% B for 5 min and return to initial conditions in 2 min. Injection volume was 5 µl. Data acquisition was performed with a TripleTOF 5600 System (AB SCIEX). Data were acquired using an ionspray voltage floating (ISVF) 2800 V, curtain gas (CUR) 20, interface heater temperature (IHT) 150, ion source gas 1 (GS1) 20, declustering potential (DP) 85 V. All data were acquired using information-dependent acquisition (IDA) mode with Analyst TF 1.5 software (AB SCIEX). For IDA parameters, a 0.25-s MS survey scan in the mass range of 350–1,250 Da was followed by 50 MS/MS scans of 50 ms in the mass range of 100–1,500 (total cycle time: 2.8 s). Switching criteria were set to ions with a mass to charge ratio (m/z) greater than 350 and smaller than 1,250 with a charge state of 2–5 and an abundance threshold of more than 90 counts (cps). Former target ions were excluded for 20 s. IDA rolling collision energy (CE) parameter script was used to automatically control the CE. MS and MS/MS data obtained for individual samples were processed using Analyst TF 1.5.1 Software (AB SCIEX). Raw data file conversion tools generated mgf files, which were also searched against the UniProtKB/SwissProt database with human taxonomy restriction (ID 9606), containing 40,478 protein coding genes and their corresponding reversed entries using the Mascot Server v. 2.4 (Matrix Science). Search parameters were set as follows: methylthiol cysteins and oxidized methionines as variable modification. Peptide mass tolerance was set to 50 ppm and 0.5 Da for fragment masses, and one missed cleavage was allowed. False discovery rate (FDR ≤ 1% at peptide level) for peptide identification was manually calculated. Visualization of disjoint and overlapping protein datasets was carried out by drawing a Venn diagram of the five protein datasets using an R package.

Additional details of Methods

Quantitative real-time PCR (qRT–PCR)

qRT–PCR was performed on DNase-treated RNA using the SuperScript III Platinum One-Step Quantitative RT-PCR System (cat. no. 11732-088, Invitrogen, Life Technologies) according to the manufacturer's directions on a 7300 Sequence Detector System (Applied Biosystems). 150 ng of RNA extracted from 2.5×10^8 exosomes was used as qPCR input. Primers for human $KRAS^{G12D}$ mRNA and human $KRAS^{G12V}$ mRNA (both Sigma-Aldrich) were designed as reported previously. Briefly, the altered base in the $KRAS^{G12D}$ and $KRAS^{G12V}$ mutations was kept at the 3′ end of the forward primer. An additional base mutation was included two positions before the *KRAS* mutation to increase the specificity of the amplification of the mutant *KRAS* allele. Forward primer sequence for $KRAS^{G12D}$ mRNA: F-5′-ACTTGTGGTAGTTGGAGCAGA-3′. Forward primer sequence for $KRAS^{G12V}$ mRNA: F-5′-ACTTGTGGTAGTTGGAGCAGT-3′. Forward primer sequence for *KRAS* wild-type mRNA: F-5′-ACTTGTGGTAGTTGGAGCTGG-3′. Reverse primer for all *KRAS*: R-5′-TTGGATCATATTCGTCCACAA-3′. Human-specific *GPC1* mRNA primer pairs (cat. no. PPH06045A) and 18S RNA primer pairs (cat. no. QF00530467)

were purchased as ready-to-use specific primer pairs from Qiagen. For murine *Gpc1* mRNA analysis, the following primer pair was used: forward primer sequence mmu_GPC1 mRNA 5′-CAGCGAA GTCCGCCAGAT-3′ and reverse primer sequence mmu_GPC1 mRNA 5′-CAGACCTCCCGAGTGCTAGG-3′. For murine *Kras* analysis, the following primers were used: forward primer sequence for $Kras^{G12D}$ mRNA: F-5′-ACTTGTGGTGGTTGGAGCAGC-3′. Reverse primer sequence for $Kras^{G12D}$ mRNA: F-5′-TAGGGTCATACTCATCCACAA-3′. Forward primer sequence for *Kras* wild-type mRNA: F-5′-ACTTGTGGTG GTTGGAGCTGG-3′. Reverse primer sequence for *Kras* wild-type mRNA: 5′-TTGTGGATGAGTATGACCCTA-3′. Mouse primers for 18S RNA were purchased as ready-for-use specific primer pairs (cat. no. AM1716, Ambion). Threshold cycle (C_t), the fractional cycle number at which the amount of amplified target reached a fixed threshold, was determined and expression was measured using the $2^{-\Delta Ct}$ formula, as previously reported. In Fig. 2d and Extended Data Fig. 2b, raw C_t values were presented. When the specific RNA product could not be detected by qPCR ("undetermined"), the C_t value was set to 0.

Flow cytometry sorting of exosome-bound beads

Exosomes were attached to 4-µm aldehyde/sulfate latex beads (Invitrogen) by mixing at least 4.0×10^8 exosomes in a 10-µl volume of beads for 15 min at room temperature with continuous rotation. The same number of exosomes from all groups was analysed for each independent study cohort. This suspension was diluted to 1 ml with 1× PBS and left for 30 min rotating at room temperature. The reaction was stopped with 100 mM glycine and 2% BSA in 1× PBS and left rotating for 30 min at room temperature. Exosome-bound beads were washed once in 1× PBS/2% BSA and centrifuged for 1 min at 10,000 r.p.m., blocked with 10% BSA with rotation at room temperature for 30 min, washed a second time in 1× PBS/2% BSA and centrifuged for 1 min at 10,000 r.p.m., and incubated with anti-GPC1 (PIPA528055, Thermo Scientific; 3 µl of antibody in 20 µl of 2% BSA/1× PBS) during rotation for 30 min at 4 °C. The specific detection of GPC1 on the exosomes derived from the serum of patients with pancreatic cancer using multiple antibodies has been validated independently (https://www.biorxiv.org/content/10.1101/145706v3). Beads were centrifuged for 1 min at 10,000 r.p.m., the supernatant was discarded and beads were washed in 1× PBS/2% BSA and centrifuged for 1 min at 10,000 r.p.m. Alexa-488 secondary antibody (Life Technologies; 3 µl of antibody in 20 µl of 2% BSA/1× PBS) was used for 30 min with rotation at 4 °C, after which beads were washed three times in 1× PBS/2% BSA. Secondary antibody incubation alone was used as control. Beads with $GPC1^-$ and $GPC1^+$ bound exosomes were sorted from the lowest and highest edges of the fluorescence signals, respectively. With this approach, samples were enriched for beads with either mainly positive or mainly negative exosomes. Next, sorted samples were treated with heat cycles (6 times at 37 °C with PBS washes in between (Landsteiner Heat)) to inactivate the antibodies. A one-pulse sonication (20 kHz) was used to release exosomes from the beads. The solution was centrifuged at 12,000*g* for 1 min to pellet the beads. Exosomes (separated in a GPC1-positive fraction and a GPC1-negative fraction) were recovered from the supernatant by ultracentrifugation at 150,000*g* at 4 °C overnight. The exosomes pellet was washed with 11 ml of 1× PBS, followed by a second step of ultracentrifugation at 150,000*g* at 4 °C for 2 h. Afterwards, the supernatant was discarded. Exosomes used for TEM were fixed and underwent immunogold labelling as described in the Methods section "Immunogold labelling and electron microscopy." Exosomes used for qRT–PCR were lysed in Trizol as described in the Methods section "RNA extraction of cells and exosomes." Subsequently, qRT–PCR was performed as described in Methods section "Quantitative real-time-PCR (qRT–PCR)."

Supplementary information is available in the online version of this Amendment.