Supplements

Materials and methods

Immunogold assay

Exosomes were fixed in 2% (w/v) paraformaldehyde and later loaded onto formvar-coated nickel grids (TAAB Laboratories Equipment Ltd.) and dried thoroughly at room temperature. After rinsing with PBS 3 times, the grids were then incubated with 0.05 M glycine/PBS for 40 min to quench free aldehyde groups and permeabilized with 0.2% (v/v) Triton X-100/PBS for 5 min. Grids were then washed in PBS 3 times with each wash lasting for 10 minutes. In order to prevent nonspecific binding, samples were blocked with PBS containing 5% (w/v) BSA for 30 min, then washed twice in incubation buffer (0.2% (w/v) BSA in PBS) for 5 min and incubated overnight at 4°C with 50 μg/ml anti-CD63 (Genetex) antibody in PBS/0.5% BSA. After primary antibody incubation, samples were then washed and stained with gold-labelled secondary goat anti-rabbit antibody (Aurion) for 2 hours at room temperature and postfixed with 1% glutaformaldehyde for 10 min. After secondary antibody incubations, grids were washed in milli-Q water for 10 min, three times and contrasted in 1% filtered uranyl acetate solution for 5 min. Grids were stored when fully dried and imaging is performed using a Hitachi H-7100 transmission electron microscope equipped with a Gatan 832 digital camera (Gatan Inc.) at an acceleration voltage of 100 KV.

ELISA

Levels of TGF-β1 in plasma or in exosome were quantified employing a Quantikine ELISA kit as described by the manufacturer (R&D Systems). For each assay, 10 ul of exosomes was used. Prior to verifying the levels of TGF-β1 in plasma and in exosomes, samples were first acidified to activate latent TGF-β1 with 1 N HCl for 10 minutes at room temperature and neutralized with an equal volume of 1.2 N NaOH and 0.5 M HEPES.

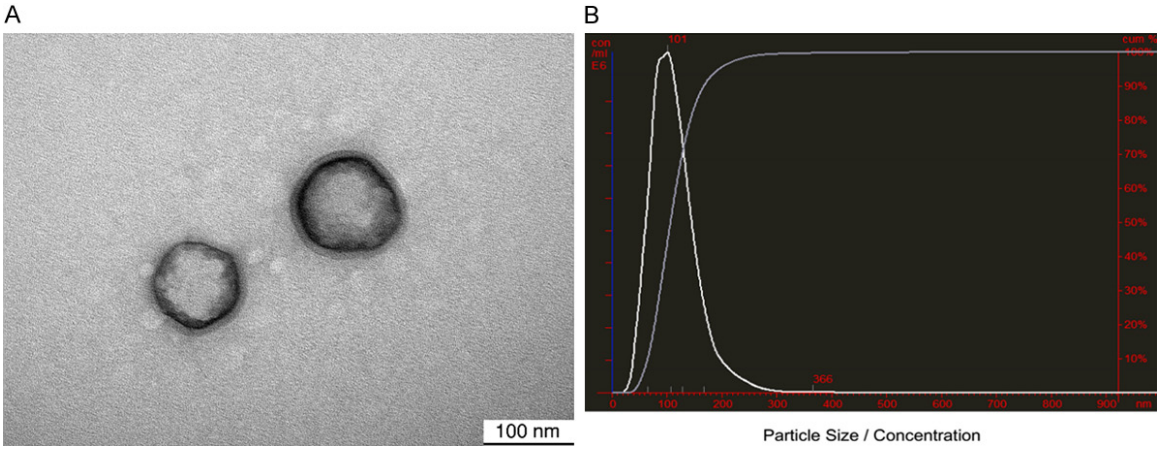
Supplementary results

The expression of TGF-β in gastric cancer

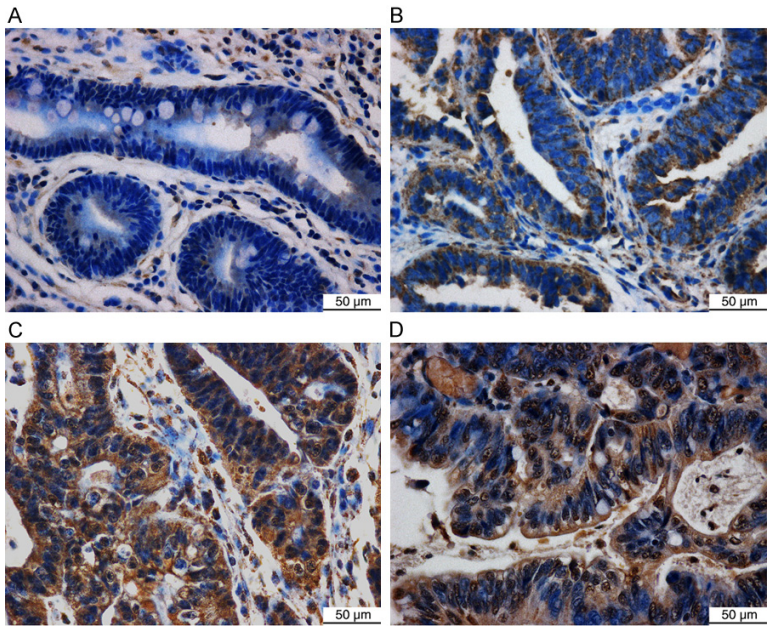
A preliminary CDNA microarray was performed to investigate gene expression in 6 paired tumor and non-tumorous tissue of gastric cancer. Among the 255 genes identified, 12 genes were found to be up-regulated, while 13 genes were down-regulated in tumors (Supplemental Table 1). TGF-β1, which encode transforming growth factor beta 1, was one of the up-regulated genes in gastric cancer tissues, especially in those of advanced stages. Immunohistochemical staining of TGF-β in paraffin-embedded GC tissues were performed tovalidate the microarray finding. The non-tumorous gastric epithelium showed little or no TGF-β expression. The tumorous part of GC expressed obvious TGF-β staining, and the staining intensities of TGF-β in tumors increased with the pathological stages of GC (Supplementary Figure 2).

Supplementary Table 1. Differentially expressed genes in gastric cancer tissue-microarray analysis

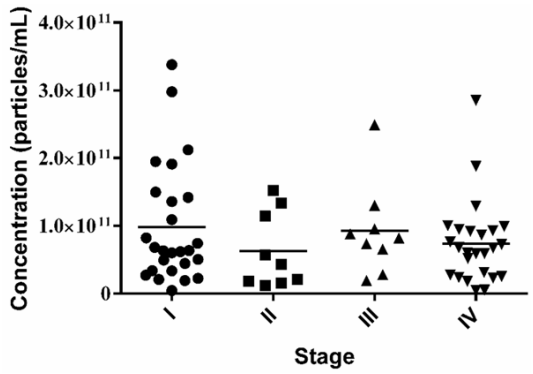
Up-regulated	Down-regulated
C1QB	CDC42
C1R	HSH2D
C1S	IL10RB
C2	IL13
HIF1A	IL1B
IL18	IL9
LIMK1	MAP2K1
RIPK2	MAP3K9
STAT1	MAPKAPK2
TGFβ1	MX2
TLR7	OASL
TREM2	PIK3C2G
	TLR3



Supplementary Figure 1. EM picture and NTA analysis of the isolated exosomes of GC patients. A. Representative electron microscopic image of isolated exosomes at 150,000× magnifications. These particles exhibited a spherical, membrane-bound characteristic. Scale bar represents 100 nm. B. Representative NTA plot showed the size and concentration distribution of particles in the plasma of GC patients, which shows a peak diameter of 101 mm.



Supplementary Figure 2. Representative immunohistochemical staining of TGF-β1 in gastric cancers. (A) Stage I; (B) Stage II; (C) Stage III; (D) Stage IV. Bars represent 50 μm.



Supplementary Figure 3. The amount of exosomes released in different stages of gastric cancer. There was no difference between groups (p=0.4971).