Human mast cells release extracellular vesicle-associated DNA
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Extracellular vesicles (EVs) carry multiple bioactive molecules, including proteins and nucleic acids. Cell-free, extracellular DNA has been reported to be present in cell cultures and in multiple body fluids, but its relative location in relation to EVs has not been described. This study demonstrates that DNase-sensitive nucleic acids are present on the surface of EV isolates. Association of EV-DNA was revealed by increase of EV zeta potential and particle number upon DNase treatment. Additionally, cells exposed to EVs with associated DNA show the presence of cytoplasmic DNA traces intracellularly. In conclusion, we suggest that DNA can be associated to the surface of EVs and can be taken up by recipient cells. DNA on EV surfaces may influence their function in recipient cells.

Objective
This work aims to determine the relative location of cell-free DNA in relation to extracellular vesicles.

Introduction
Extracellular vesicles (EVs) are membrane bound structures released by cells, and found in all body fluids. The populations of EVs are diverse, and the nomenclature includes terms such as exosomes, ectosomes and microvesicles. Previously, it has been shown that the subpopulations of EVs called exosomes can carry both mRNA and microRNA, which can mediate function in recipient cells [Valadi 2007[1]]. Additionally, dsDNA has previously been proposed to be associated with EVs [Kahlert 2014[2], Lee 2014[3], Balaj 2011[4], Thakur 2014[5], Guescini 2009[6]], but its relative location to/in EVs has not been well described. This study reports for the first time that cell-free DNA can be associated with the outside of EVs, which may influence aggregation of these EVs.
DNA is associated with extracellular vesicles. (A) Isolated EVs, and EVs were floated on an optiprep gradient and treated with DNase-I or RNAse prior to DNA isolation (apoptotic bodies and microvesicles had been removed at 16000g). Nucleic acids were visualized with agarose gel electrophoresis labeled with nucleic acid stain (GelStar). The isolated EVs were also analyzed with NTA (Zetaviewer®) to determine the zeta potential (B) on EVs, and the particle number (C) with or without DNase treatment. Isolated EVs were incubated with human mesenchymal stem cells for 1 or 24 hrs, and DNA was labeled with DAPI and visualized by fluorescent microscopy at 24 hr (D), and cytoplasmic DAPI staining foci per cell were counted (E). Student’s T-test was used to determine significant differences between groups (* p < 0.05. N = 3).
Results and discussion

RESULTS:
On a nucleic acid-stained gel, clear DNA bands were visualized, and these were present not in samples pretreated with DNase-I but in samples treated with RNase (Figure 1A). The EVs fraction isolated from the density gradients had a charge of $-70 \text{ mV}$, which was increased to $-50 \text{ mV}$ by DNase-I treatment (Figure 1B). This result indicates that DNA is present on the outside of the EVs, and this DNA contributes to the negative charge of the vesicles. Further, DNase treatment increased the number of particles measured by nanoparticle tracking analysis, suggesting that the DNA to a certain degree contributes to aggregation of the isolated EVs (Figure 1C). As a biological readout, isolated EVs associated with extracellular DNA were taken up by human mesenchymal stem cell in a time-dependent manner (Figure 1D and E).

DISCUSSION:
EVs carry multiple bioactive molecules, including proteins, various RNA species, and according to the present study, DNA. In our case, the DNA was observed in EV isolates from a human mast cell line. Specifically, our study argues that the DNase-sensitive nucleic acids are present on the outside of the EVs. Furthermore, the EV-associated DNA can be taken up by recipient cells, which could alter cellular responses.

It is known that EVs can mediate an array of biological messages to recipient cells—including surface-to-surface antigen presentation and receptors activation—and can deliver RNA (e.g. mRNA, miRNA) cargo to recipient cells. However, the presence and function of DNA as a cargo on the outside of EVs is less explored. EVs have previously been associated with cell-free DNA that carries retrotransposon elements and oncogenes, but overall EV-associated DNA has been extensively characterized. A recent report emphasizes the presence of dsDNA inside of the EVs, whereas we find that a majority of DNA from the human mast cell line is associated with outer perimeter of EVs, since it is sensitive to DNase treatment without lysing the EVs. Our study also indicates, for the first time, that DNA covering floated EVs can lead to an increase in the net negative charge of the vesicles. This was confirmed by reduction of net negative charge from EVs by DNase treatment. We were also able to monitor the increase in particle numbers after DNase treatment, indirectly suggesting that EV-DNA may lead to aggregation of EVs. These results are in line with previous observations made in various electron micrographs, showing clustering of EVs, which could have occurred because of DNA on the surface of these vesicles. The aggregation of EVs may be secondary to nonspecific aggregation of EVs during ultracentrifugation, but our study suggests that EV-related DNA can contribute to this observation. As we observed that the extracellular DNA floated in the density gradient, we argue strongly that it is associated with the floating vesicles with relatively low density. Also, it is has been shown that exogenous plasmids DNA if associated with EVs are taken up more efficiently in recipient cells than free DNA, again arguing that association to EVs could be involved in DNA uptake. Similarly, we also observed a time-dependent increase in cytoplasmic DNA foci in EV treated recipient human mesenchymal stem cells. Overall, this study highlights the need to define the EV-associated DNA in delivering biological function in cells that take up these EVs.

Conclusions
We conclude that cell-free DNA can be associated with the outside of EVs, which can cause aggregation of these EVs, possibly influencing the effects of EVs in recipient cells.

Limitations
This work is based on cell-line-produced EVs, which may not reflect a biological function in vivo.

Conjectures
In the future, the detailed nature of the EV-associated DNA needs to be determined, in relation to sequences and intracellular origin. Also, the role of the EV-associated DNA in recipient cells may be studied.
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**Methods and supplementary material**

Please see https://sciencematters.io/articles/201602000034.

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**Ethics Statement**

Not applicable. No animal was used in the experiments. Therefore, no ethical approval is required.

**Citations**


