Circulating extracellular vesicles (EVs) as biomarkers for gastrointestinal stromal tumors (GISTs)

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Abstract

Background: Gastrointestinal stromal tumors (GISTs) are mesenchymal neoplasms that arise in the gastrointestinal tract. GISTs are differentiated from other types of Soft Tissue Sarcoma (STS) on the basis of CD34, CD117, PDGFRA and DOG1 expression used by the pathologist in immunohistochemistry (IHC) on tumor biopsies. Extracellular vesicles (EVs) are spherical nano-vesicles which are released from almost all living cells. These EVs carry biological information from their parental cells and play a role in intracellular communication. We assessed EVs characteristics from tumor cells and patient's blood samples as a tool to determine GIST pathology. **Methods:** First, EVs were isolated from human GIST and STS cell lines as an in vitro disease model. These EVs were characterized by size, concentration and membrane antigens for CD117, CD34, PDGFRA and DOG1 using nanoparticle tacking analysis (NTA) and Fluorescence-activated cell sorting (FACS). Next, EVs were isolated from blood samples of GIST and STS patients as well as healthy controls and characterized as described above. **Results**: EVs were found to be similar by size in GIST and STS cell lines but with lower concentration in GIST cells. Higher levels of CD117 were found on EVs from all cell lines, however the expression of CD34 and DOG1 were higher on GIST EVs compared to the STS cells EVs. Circulating EVs isolated from plasma of GIST patients were smaller in size and with higher concentration than circulating EVs isolated from STS patients. This GIST circulating EVs expressed higher levels of DOG1 compared to STS circulating EVs.

Results

Isolation and characterization of human GIST-T1 cells derived EVs

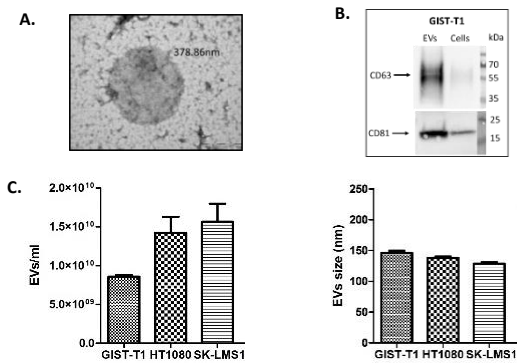

Figure 1. (A) EVs were isolated from GIST-T1 human cells and analyzed by transmission electron microscopy (TEM). (B) Western blot analysis of the EVs markers CD81 and CD63. (C) Concentration (left) or size distribution (right) of EVs isolated from human GIST-T1 cells, SK-LMS1 human leiomysarcoma cells and HT1080 human fibrosarcoma cells were determined by NTA.

GIST-T1 cells derived EVs express higher levels of DOG1 and CD34 markers compared to STS cells

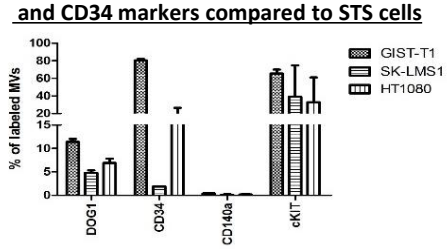

Figure 2. EVs were isolated from human GIST-T1 cells, human SK-LMS1 leiomysarcoma cells and HT1080 human fibrisarcoma cells and analyzed by FACS analysis using specific fluorescent antibodies for DOG1, CD34, CD140a (PDGFRA) and cKIT markers.

Results

Isolation and characterization of circulating blood EVs from GIST and STS patients

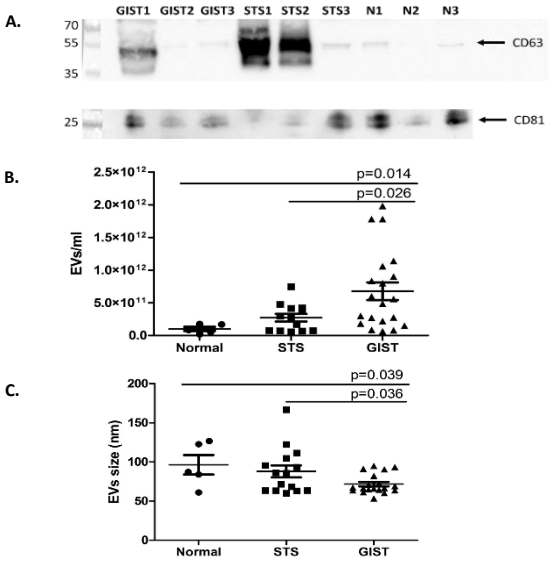

Figure 3. (A) EVs were isolated from plasma of GIST patients (GIST1, GIST2 and GIST3), STS patients (STS1, STS2 and STS3) as well as healthy controls (N1, N2 and N3) and analyzed by Western blot for EVs markers CD81 and CD63. (B) Concentration or size distribution (C) of EVs isolated from plasma of 15 STS patients, 20 GIST patients and 6 healthy controls were determined by NTA

Circulating blood EVs from GIST patients express higher levels of DOG1 compared to STS patients

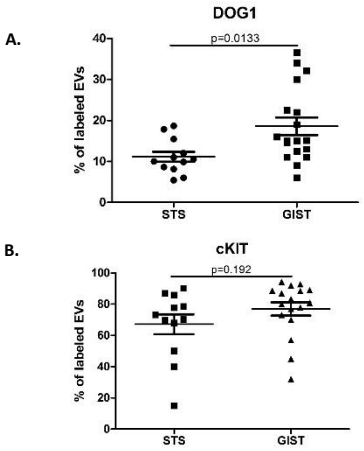

Figure 4. EVs were isolated from plasma of 15 STS patients and 20 GIST patients and analyzed by FACS analysis using specific fluorescent antibodies for DOG1 (A) and cKIT (B).

Conclusions

Our results demonstrate the potential use of circulating EVs as biomarkers for GISTs. Specifically the use of EVs expressing DOG1 to differentiate GISTs from other types of STS. Such a tool may replace the need for tissue biopsy in patient diagnosed with SOL of unknown pathology using a simple blood test. Also, such EVs may be used as a tool for patient oncological follow-up and to evaluate treatment response and disease recurrence.