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ORAL ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Platelets Sequester Extracellular DNA, Capturing Tumour-Derived and Free Fetal DNA

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Introduction & Hypothesis

Platelets are small, multi-functional cells that lack a nucleus but contain RNA and translational machinery for protein synthesis. Platelet RNA mostly derives from parent megakaryocytes, but they also sense and sequester endogenous and pathogen-derived nucleic acids during circulation (D'Ambrosi, 2021; Koupenova, 2019). Nucleated cells release DNA after cell death or aberrant mitosis, resulting in 'cell-free' DNA in plasma (cfDNA). Excess cfDNA is deleterious. cfDNA isolated from platelet-poor plasma is emerging as a major liquid biopsy tool in cancer and antenatal screening, but a major limitation is its low abundance, especially in early-stage disease.

Given their role in sensing pathogen-derived nucleic acids, we hypothesized that platelets may clear cfDNA from plasma, and that clinically-relevant insights may be derived from the analysis of DNA fragments contained in platelets.

Methods

Live/fixed imaging using specific DNA probes and fluorescence in-situ hybridization (FISH), droplet digital PCR (ddPCR), NGS and flow cytometry were applied to platelets isolated from Streck, EDTA tubes or apheresis bags from healthy donors,

pregnant women, patients with cancer and murine platelets from cancer models. DNA was extracted from platelet pellets or platelet-depleted plasma (cfDNA) using the Qiagen QIAamp Circulating Nucleic Acid Kit.

Results

Using live cell imaging and flow cytometry, significant DNA content (Draq5+) was detected in ~8% of platelets from healthy donors, including in platelets with low RNA content (SYTO-13-negative). Overall Draq5+ platelets were larger and primed for agonist activation. FISH and ddPCR of platelets from pregnant women carrying male fetuses detected Y-chromosome fragments (n=10), confirming that platelet DNA is not solely derived from parent megakaryocytes but also sequestered during circulation (Fig. 1a). In contrast, Y-chromosome genes were not detected in DNA isolated from mononuclear cells or red blood cells pellets. Acute platelet depletion in an ITP mouse model (n=20) led to a >2-fold increase in cfDNA extracted from platelet-poor plasma, suggesting a role for platelets in plasma DNA clearance.

Platelet uptake *in vitro* was rapid, visible using live cell microscopy within 2 minutes of co-culture with cancer cells labelled with a probe that irreversibly intercalates to nuclear DNA. ddPCR of platelet DNA following co-culture of healthy donor platelets with cancer cell lines detected a range of canonical cancer driver mutations, including in *PI3K*, *BRAF* and *JAK2*. DNase treatment of co-cultured platelets did not reduce mutant allele content, indicating that platelets encapsulate DNA and protect it from degradation.

We explored the mechanism of platelet DNA uptake. Platelets internalized DNA contained in extracellular vesicles (EVs). Platelet uptake of "free" DNA was also confirmed, with uptake of synthetic DNA fragments of varying lengths (120 bp to 650 bp). Importantly, platelet DNA detection was enhanced following exocytosis inhibition, confirming that platelet DNA uptake and release is an active process.

Whole genome sequencing revealed that platelets contain a repertoire of DNA fragments that map across the nuclear genome, similar to cfDNA, and that the majority of platelet DNA is of nuclear not mitochondrial origin. We also found tumor-derived DNA showing multiple copy number aberrations in a patient with pancreatic carcinoma.

In a transgenic mouse model of colorectal cancer, mutant *KRAS*G12D alleles were readily detectable in platelet DNA and, notably, in higher abundance in platelets than platelet-poor plasma (cfDNA) in 11/16 mice (Fig. 1b). Similarly, *KRAS*G12D was detected in platelets of mice with orthotopic pancreatic adenocarcinoma (n=4).

Finally, to explore utility in cancer screening, we analysed patients with high-risk, pre-malignant colonic lesions (serrated polyps). Remarkably, the driver mutation *BRAF*V600E was detected in platelets in 16% patients despite the small-size lesions (5/30, Fig.1b).

Conclusions

This study establishes a role for platelets in sequestration of cfDNA, an aspect of platelet biology that has not previously been highlighted and is of substantial clinical relevance. Their abundance, ease of isolation, and continuous tissue perfusion make platelets ideal 'sentinels' for genetic perturbations including in early stage/pre-malignant disease.

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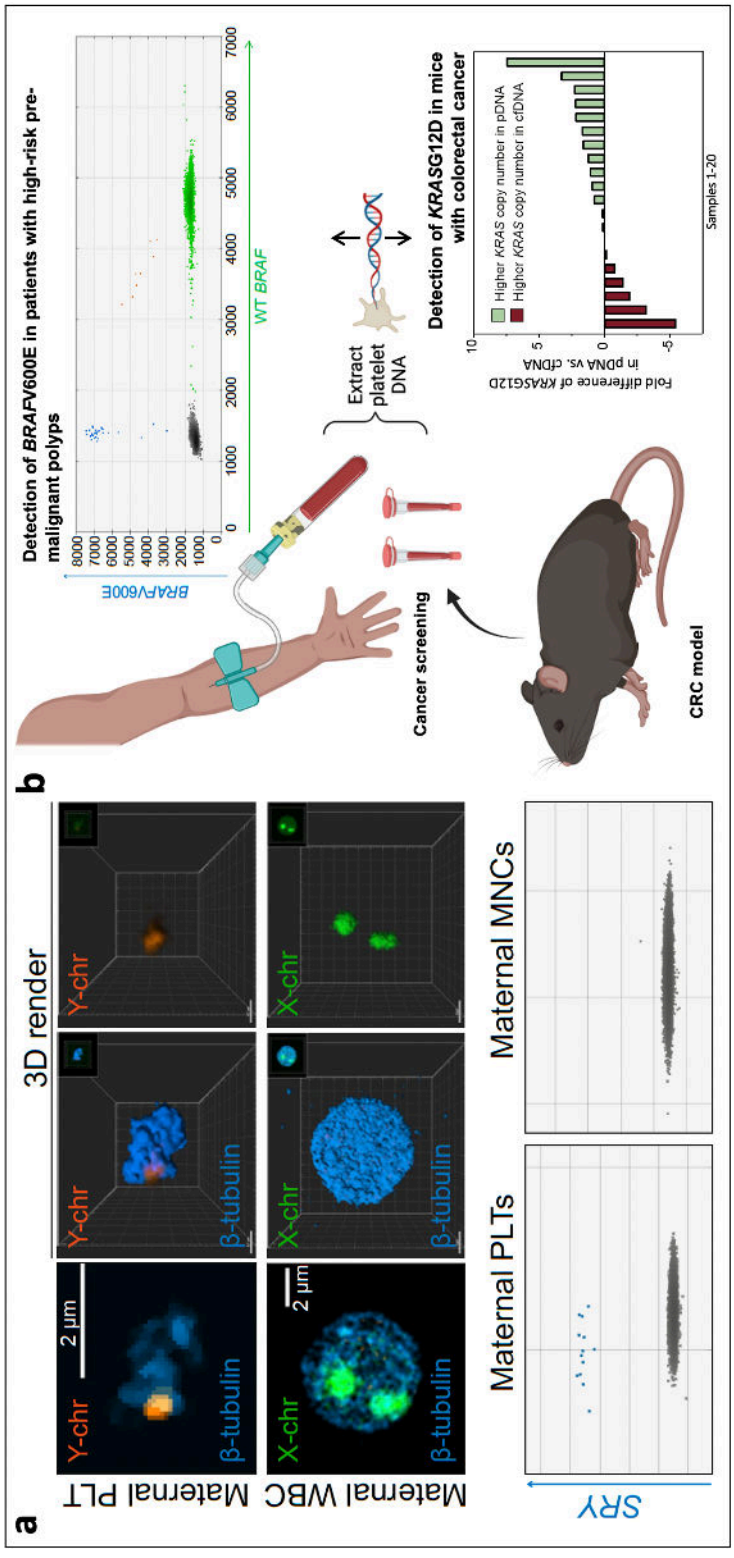

Fig 1: a, Fluorescence in-situ hybridization chromosome paint and droplet digital PCR (ddPCR) showing detection of the Y-chromosome gene SRY in maternal platelets, but not mononuclear cells (MNCs) sampled from mothers of male neonates prior to delivery. Platelets and MNCs were counterstained with β-tubulin (blue) and imaged using a ZEISS LSM900, 63 x magnification. Representative images shown. **b,** Schematic showing the isolation of platelet DNA from patients undergoing cancer screening and mice modelling colorectal cancer. Top right is a representative ddPCR plot showing the number of copies of *BRAF*V600E (blue) detected in pDNA in 2 patients with SSLs. Bottom right is a waterfall plot showing the fold difference in copies of *KRAS*G12D detected per µl of DNA for pDNA vs. cfDNA. Image created with BioRender.com. Abbreviations: cell-free DNA (cfDNA); colorectal cancer (CRC); mononuclear cells (MNCs); platelet DNA (pDNA); platelet (PLT); white blood cell (WBC); wild- type (WT); X chromosome (X-chr); Y chromosome (Y-chr)

Figure 1