Received: 23 February 2021 | Accepted: 27 September 2021

DOI: 10.1111/jth.15543

ORIGINAL ARTICLE

Uptake of platelets by cancer cells and recycling of the platelet protein CD42a

Nadine Martins Castanheira[1] | Anna K. Spanhofer[1] | Sebastian Wiener[1] | Stefanie Bobe[1,2] | Hermann Schillers[1]

[1]Institute of Physiology II, University of Muenster, Muenster, Germany

[2]Gerhard-Domagk-Institute of Pathology, University Hospital Muenster, Muenster, Germany

Correspondence
Hermann Schillers, Institute of Physiology II, University of Muenster, Robert-Koch-Str. 27b, 48149 Muenster, Germany.
Email: Hermann.Schillers@uni-muenster.de

Funding information
MedK Muenster; Cells-in-Motion Cluster of Excellence, Grant/Award Number: EXC 1003–CiM

Abstract

Background: It is well accepted that the bidirectional crosstalk between platelets and cancer cells promotes tumorigenesis and metastasis. In an early step, cancer cells trigger platelet granule and extracellular vesicle release that is needed to facilitate cancer cell survival in circulation.

Objectives: To discover the early crosstalk of cancer cells and platelets.

Methods: Cancer cells were incubated with freshly isolated and stained human platelets. Confocal laser scanning microscopy and flow cytometry was used to visualize and to quantify platelet uptake and the membrane presence of CD42 on cancer cells. Dyngo4a was used to test if platelet uptake is a dynamin-dependent process.

Results: We found a dynamin-dependent uptake of platelets by cancer cells. This is followed by the recycling of the platelet-specific protein CD42a and its incorporation into cancer cells' plasma membrane, which is not a result of platelet RNA transfer by platelet-derived microparticles and exosomes. Time course of platelet uptake follows a sigmoid function revealing that 50% of the cancer cells are positive for platelets after approximately 38 min. Platelet uptake was observed for the tested cancerous cells (A549, MCF-7, and MV3) but not for the non-cancerous cell line 16HBE14o−.

Conclusions: Our results demonstrate that cancer cells hijack platelets by phagocytosis and recycling of platelet membrane proteins. The uptake of platelets has additional advantages for cancer cells: access to the entire and undiluted platelet proteome, transcriptome, and secretome. These novel findings will allow further mechanistic elucidation and thus help us gain deeper insights into platelet-assisted hematogenous metastasis.

KEYWORDS
dynamin, flow cytometry, metastasis, phagocytosis, recycling

Manuscript handled by: Matthew T. Rondina

Final decision: Matthew T. Rondina, 27 September 2021

1 | INTRODUCTION

Most cancer deaths are related to metastasis of distant organs via dissemination of cancer cells through blood or lymphatic vessels. Circulating tumor cells (CTCs) are cancer cells that have shed from the primary tumor and entered circulation. These CTCs are exposed to immune surveillance and have to attach to the vessel wall to form a metastatic lesion.[1] It is well accepted that platelets have a pro-metastatic function when interacting with CTCs.[2] The common view of platelet–CTC interaction is an activation of platelets upon contact to CTCs (tumor cell-induced platelet-aggregation, TCIPA)[3] followed by aggregate formation and/or formation of a so-called "platelet cloak" around cancer cells.[4,5] Platelet cloaks serve as a physical barrier[6] to protect cancer cells from immune surveillance[7] and facilitate the continued adhesion of cancer cells to the vessel wall, extravasation, and metastasis.[8] Upon activation, platelets release a multitude of mediators that regulate cancer cell activities, which include growth factors, cytokines, matrix metalloproteinases (MMPs), biogenic amines, and many others (for a review see Li[2]). Most of these mediators act on cancer cell surface receptors but there is also an intercellular transfer of bioactive molecules between platelets and cancer cells.

Platelets and cancer cells interact reciprocally.[9] In cancer patients, platelets actively absorb tumor-derived extracellular vesicles harboring proteins, RNA molecules, and surface membrane proteins. These platelets, called tumor-educated platelets (TEPs),[10] represent a target for liquid biopsies to diagnose several types of cancer.[11] Conversely, the transfer from platelets to cancer cells depends mainly on platelet-derived extracellular vesicles. Platelet-derived microparticles (PMPs; vesicles of 0.1–1 μm) are generated from the platelet plasma membrane and released upon activation.[12] PMPs mediate the intercellular transfer of lipids, surface receptors, and even enzymes[13] but also messenger RNAs (mRNAs), a variety of microRNAs (miRNA), and a repertoire of RNA processing proteins.[14] Exosomes, which are also derived from platelets, are spherical fragments (30–100 nm) that originate from multi-vesicular bodies upon platelet activation.[15] Platelet-derived exosomes are also carriers for proteins and RNA, interacting with target cells by membrane fusion, receptor-ligand interaction, or endocytosis by phagocytic mechanism.[16] Platelet-derived exosomes and PMPs mediate changes in the recipient cell functions by changing the gene expression of cancer cells and promote metastasis.[17]

A transfer of platelet membrane proteins to cancer cells by membrane fusion and transmembraneous integration of these proteins was shown for major histocompatibility complex (MHC) class I. This rapid utilization of platelet proteins protects cancer cells from immune surveillance immediately after intravasation.[18]

Among these different strategies platelets unfold their protumorigenic and prometastatic effects, another way of transfer between platelets and cancer cells remains almost unnoticed: the phagocytic uptake of platelets.

Phagocytosis of platelets by macrophages,[19] neutrophils,[20] and hepatocytes[21] in the reticuloendothelial system is a clearance mechanism to maintain normal physiological blood platelet counts. Phagocytosis is not only used by immune cells but also by "non-professional phagocytes" like endothelial cells[22] and epithelial cells.[23,24] Phagocytosis of platelets was shown for human umbilical vein endothelial cells (HUVEC),[25] hemangioendothelioma cells (EOMA), and mouse brain microvascular endothelial cells.[26]

In this work we investigated the platelet uptake by cancerous and non-cancerous cells and the fate of platelet proteins with CD42a (GPIX).

Essentials

- Cancer cells phagocytose platelets in a dynamin-dependent way.
- Cancer cells recycle the platelet protein CD42a to its own plasma membrane.
- Fifty percent of the cancer cells are positive for platelet fragments after 38 min.
- Platelet uptake was observed for the tested cancerous cell lines A549, MCF-7, and MV3.
- No platelet uptake was observed for the tested non-cancerous cell line16HBE14o^{-}.
- Phagocytosis of platelets enables access to their undiluted proteome, transcriptome, and secretome.

2 | MATERIALS AND METHODS

2.1 | Cell culture

The non-small-cell lung cancer cell line A549 (RRID:CVCL_0023) was cultured in DMEM medium (Dulbecco's modified eagle's medium–high glucose; Sigma-Aldrich), the immortalized lung epithelial cell line 16HBE14o− (RRID:CVCL_0112) was grown in Eagle's Minimal Essential Medium (Invitrogen) supplemented with 2 mM L-glutamine, and the mammary ductal carcinoma cell line MCF-7 (RRID:CVCL_0031) and the amelanotic melanoma cell line MV3 (RRID:CVCL_W280) were grown in RPMI 1640 Medium FG (1215; Sigma-Aldrich). All culture media were supplemented with 10% fetal bovine serum (PAA Laboratories), 50 U/mL penicillin, and 50 μg/mL streptomycin and cultured in an incubator at 37°C and 5% CO_2.

2.2 | Isolation of human platelets from peripheral blood

All blood donors gave their written informed consent in accordance with the Declaration of Helsinki. Venous blood (5 mL) was drawn in a citrate S-Monovette (Sarstedt) from healthy volunteers, who did not take any anti-coagulative drug within the

previous 2 weeks. Five hundred μL acid-citrate-dextrose buffer (39 mM citric acid, 75 mM sodium citrate, 135 mM dextrose, pH 7.4 ACD-buffer, Sigma-Aldrich) was added (1:10 [v/v]) and centrifuged for 20 min at 270 g. The supernatant of platelet-rich plasma (approximately 2 mL) was transferred with a large orifice pipette to a 13 mL tube and the same volume of platelet buffer (5 mM HEPES; 140 mM NaCl; 4 mM KCl; 1 mM $MgCl_2$; 1 mM D-Glucose; 3.5 g/L bovine serum albumin; 10 mL/L penicillin/streptomycin; and ACD-buffer [1:10, v/v)]) were added. The tube was inverted two times and centrifuged at 500 g for 10 min. The supernatant was removed and the pellet was resuspended in 2 mL of platelet buffer. Residual erythrocytes in the preparation were spun down at 100 g for 5 min. All steps were conducted at room temperature.[27]

Isolated platelets were fluorescently marked with either the fluorescein isothiocyanate (FITC)-labeled mouse anti-human CD42a antibody (Clone Beb1; BD Biosciences) or the unspecific membrane-anchoring fluorophore PKH67 (Green Fluorescent Cell Linker [Sigma-Aldrich]) according to manufacturer's protocols.

2.3 | Static incubation

Glass bottom FluoroDishes (WPI) were coated with collagen G (calf skin collagen G, Biochrom). Cells were grown to confluency and incubated with 400 μL of PKH67- or anti CD42a-FITC stained platelets (~$140{\cdot}10^6$ platelets) for different time intervals in cell culture medium at 37°C (CO_2 incubator). Unstained platelets served as control in all experiments. Immediately after the specified incubation time, FluoroDishes were washed three times with $PBS^{+/+}$ (phosphate buffered saline with Ca^{2+}/Mg^{2+}) to remove non-adherent and non-interacting platelets. Cells were fixed with 4% paraformaldehyde for 20 min in darkness at room temperature and washed two times with $PBS^{+/+}$. Plasma membrane of cells was stained with 10 μg/mL wheat germ agglutinin-Alexa Fluor 555 conjugate (Invitrogen) and the cell nuclei with DAPI.

2.4 | Dynamic incubation

Cells were detached from the cell culture flask using ethylenediaminetetraacetic acid (EDTA) buffer (0.53 mM EDTA; 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4). One hundred twenty thousand cells and ~$120{\cdot}10^6$ platelets were added in cell culture medium to non-treated 35 mm dish for suspension cultures (CytoOne®, Isogen Life Science) and placed on a tilting table (9 rpm) for different time intervals in a CO_2 incubator. Immediately after incubation cells were centrifuged (100 g, 5 min) to remove non-adherent and non-interacting platelets, fixed with paraformaldehyde and stained with wheat germ wgglutinin-Alexa Fluor 555 conjugate and DAPI. For live-cell staining of CD42a (GP IX), A549 cells were stained with primary mouse anti-CD42a (RRID: AB_2538178, Thermo Fisher Scientific) and secondary rabbit anti-mouse-Alexa 555 (red) (Abcam Inc.) antibodies prior to fixation.

2.5 | Confocal microscopy

A confocal laser scanning microscope (Leica TCS SP8) equipped with a 63× oil immersion objective was used to visualize fluorescence signals. Parameters for data acquisition (e.g., laser power, pinhole, gain etc.) were kept constant for all recordings. Leica Application Suite X (LAS X) was used for image recording and processing.

2.6 | Flow cytometry

Flow cytometry was performed with a Guava easyCyte 5 (Millipore, version 2.7) to evaluate platelet internalization by cancer cells and surface presentation of CD42a. Approximately 120,000 fixated cells were suspended in buffer and analyzed. Cells incubated with unstained platelets served as control. Flow cytometry data were analyzed using FlowJo (FlowJo LLC).

2.7 | Inhibition of phagocytosis

The highly potent dynamin inhibitor Dyngo4a ([30 μM], Abcam) was used to test if platelet uptake is a dynamin-dependent process. A549 cells were incubated with Dyngo4a for 30 min before PKH67-stained platelets were added for different time periods (60 and 90 min) at 37°C (CO_2 incubator).

2.8 | Statistical analyses

Comparisons between two groups were performed with the Mann-Whitney *U*-test. A *P*-value <0.01 was considered statistically significant. All analyses were performed in GraphPad Prism (RRID:SCR_002798) or Origin (RRID:SCR_014212). All experiments were performed with three or four biological replicates. For fluorescence activated cell sorting (FACS) analysis gated data were pooled and normalized for histogram presentation. The number of analyzed cells ranges from 101,308 to 218,329.

3 | RESULTS

We used two approaches to study platelet–cancer cell interactions: incubation of adherent cells (static) and cells in suspension on a tilting table (dynamic) with freshly isolated and stained platelets. The dynamic incubation reflects more the situation of CTCs in circulation (cells in suspension with some shear forces), albeit it is clearly not a laminar flow condition.

3.1 | Appearance of CD42a and platelet membrane in cancer cells

In contrast to the common view of formation of platelets, cloaks or cancer cell--platelet aggregates were not observed in our experiments. Instead, all experiments showed an uptake of platelets by cancer cells. Immunostaining of the platelet-specific protein CD42a (GPIX) prior to incubation with A549 cancer cells led to an appearance of CD42a fluorescence inside of A549 cancer cells. Fluorescent particles show a mainly perinuclear distribution in A549 cancer cells after 90 min of incubation under static (Figure 1A) and dynamic (Figure 1B) conditions.

To verify the platelet uptake, plasma membrane of platelets was fluorescently labeled with PKH67, a membrane anchored fluorophore with negligible cell--cell transfer, prior to incubation with A549 cancer cells. Confocal laser scanning microscopy (CLSM) revealed fluorescent particle inside A549 cytosol (Figure 2A [static], 2B [dynamic]).

Frequently, penetration of the A549 membrane by individual platelets, tube formation, and invaginations were observed indicating the uptake of whole platelets followed by fragmentation. Four representative CLSM cross sections of 30 min static incubation (Figure 3A-D) and a 3D presentation of 30 min dynamic incubation (Movie S1) illustrate this process. It was shown that phagocytosis of large structures is accompanied by formation of plasma membrane invaginations (surface-connected compartments [SCCs]) in macrophages[28] and in A549 cells,[29] which is in line with our observation. Visualization of platelets penetrating the plasma membrane of A549 indicated a phagocytic uptake mechanism.

We used Dyngo4a, a highly potent inhibitor for Dynamin-1 and Dynamin-2 to test this pathway. Dynamin-2 is a key enzyme for phagocytosis because its inhibition impairs phagocytic cup formation.[30] A549 cells were incubated with 30 μM Dyngo4a for 30 min before PKH67-stained platelets were added and Dyngo4a (30 μM) was present during incubation. No uptake of platelets was observed after 30, 60, and 90 min incubation under static and dynamic condition (Figure 4). Aggregometric tests revealed that platelet activation was not impaired by 30 μM Dyngo4a (Figure S2 in supporting information).

3.2 | Time course of platelet uptake

The time course of platelet uptake was analyzed with flow cytometry and revealed an increase of PKH67 fluorescence intensity of A549 cells upon incubation with PKH67-stained platelets for 10, 20, 60, 90 min, and 24 h (Figure 2C). Incubation with unstained platelets served as control. CLSM scans of the flow cytometry samples proved that there was no platelet attached to A549 cells. A sigmoidal fit revealed that A549 cells show the half maximal PKH67 fluorescence after 38 min and nearly all cells are positive for PKH67 after 110 min (Figure 2D).

Live-cell staining of the platelet-specific protein CD42a on the surface of A549 cells directly after dynamic incubation with PKH67-stained platelets for 90 min revealed the presence of the platelet-specific protein CD42a on the membrane of the A549 cancer cell (Figure 5A). CD42a staining of untreated A549 cells proved that native A549 cells do not express CD42a, nor in the plasma membrane (Figure S1) neither in the cytosol (Figure S3). Flow cytometry was used to analyze the CD42a expression on the membrane of A549 cells after 30, 60, and 90 min incubation with PKH67-stained platelets (Figure 5B). Histograms revealed an increase of CD42a-positive cells over this time period (Figure 5C). CLSM scans of the flow cytometry samples proved that there was no platelet attached to A549 cells. It is noteworthy that after 90 min of incubation about 45% of A549 cells exhibited CD42a

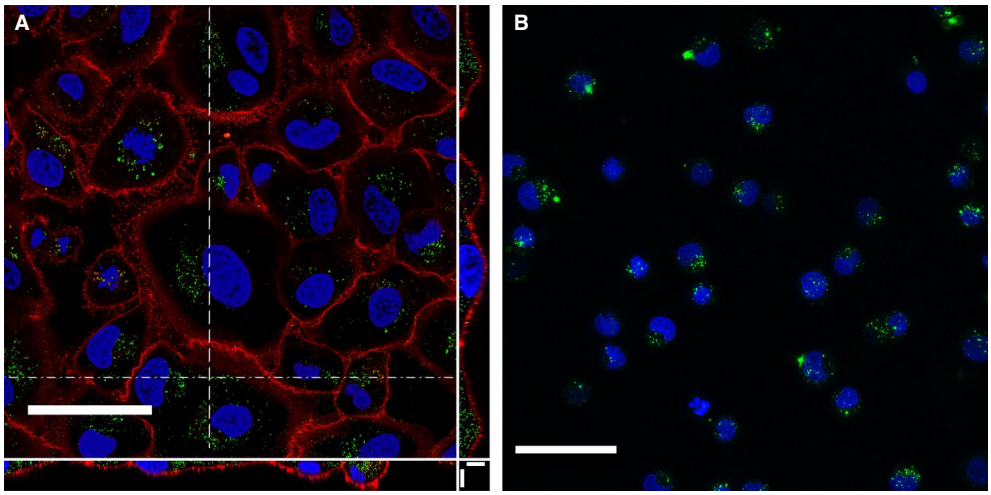

FIGURE 1 Appearance of CD42a in the cytosol of A549 cells confocal laser scanning microscopy scan of A549 cells after 90 min of incubation with CD42a-stained platelets (green) under static (A) and dynamic (B) conditions. Cell membrane in (A) was visualized using WGA-Alexa 555 (red) and cell nuclei with DAPI (blue). A: scale bar *x*/*y*: 50 μm scale bar z: 10 μm, B: scale bar 50 μm

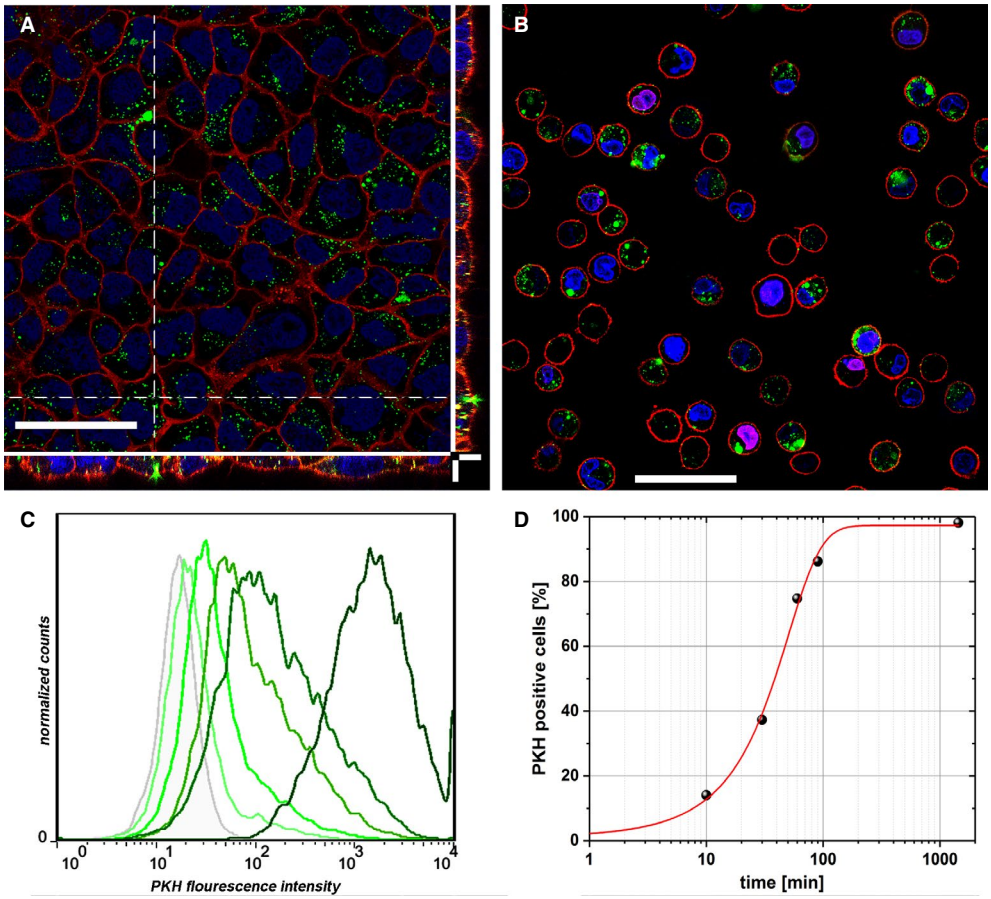

FIGURE 2 Uptake of PKH67-stained platelets in A549 cells confocal laser scanning microscopy scan of A549 cells after 90 min of incubation with PKH67-stained platelets (green) under static (A) and dynamic (B) conditions. Cell membrane was visualized using WGA-Alexa 555 (red). A: Scale bar *x*/*y*: 50 µm; z: 10 µm; B: Scale bar 50 µm. Flow cytometry analysis (A) and time-response relationship (B) of the uptake of PKH67-stained platelets under dynamic conditions. Histograms (C) show PKH67 fluorescence intensity from A549 cells without (gray) and with incubation of PKH67 stained platelets for 10, 20, 60, 90 min, and 12 h (from bright to dark green). D, The increase of A549 cells positive for PKH67, shown in a semilogarithmic scale. A dose-response fit (Levenberg-Marquardt minimization method with variable Hill slope) revealed that 50% of the A549 cells are positive for PKH67 fluorescence after 38 min

fluorescence (Figure 5C) whereas nearly all cells were positive for PKH67 fluorescence (Figure 2D).

3.3 | Platelet uptake by different cell lines

To compare the uptake of platelets between cancerous and non-cancerous lung epithelial cells we used the SV40 large T-antigen transformed, lung epithelial cell line 16HBE14o$^-$.[31] Incubation with PKH67-stained platelets for 1 h revealed an uptake of platelets in cancerous lung epithelial cells A549 but not in immortalized cells 16HBE14o$^-$ (Figure 6). There was no platelet uptake but activated platelets were found spread on the membrane of non-cancerous 16HBE14o$^-$ (Figure 6B). However, phagocytosis did not occur. An interesting feature in Figure 6A is the clear membrane staining in A549. Obviously, platelet membrane fragments are also recycled by A549 cells. Because membrane staining was not visible in 16HBE14o$^-$ cells, a cell-cell transfer of PKH67 can be excluded.

To test if the observations made with A549 cells are unique to this cell line or if this represents a characteristic for cancer cells, the highly metastatic human melanoma cell line MV3[32] and breast cancer cell line MCF-7[33] were incubated with PKH67-stained platelets under dynamic conditions. CLSM revealed an uptake of PKH67-stained platelet particles in MV3 (Figure 7A) and MCF-7 (Figure 7B) cells after 90 min of incubation with PKH67-stained platelets. Flow cytometry revealed an increase of PKH67 fluorescence intensity for MV3 (Figure 7C) and MCF-7 cells (Figure 7D). The histograms indicate a higher uptake rate for MV3 compared to MCF-7.

4 | DISCUSSION

The interactions of platelets and cancer cells are widely under investigation and have been subject to controversy in the literature. It has been discussed that platelets and the activation of platelets might play different roles in different cancers in terms

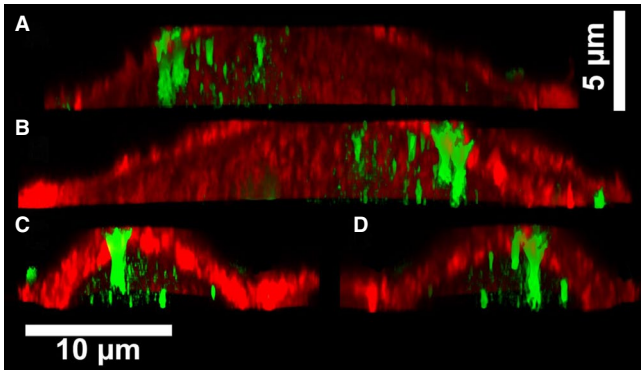

FIGURE 3 Membrane invaginations of PKH67-stained platelets in A549 cells visualized by CLSM of four representative A549 cells (A-D) after 30 min of incubation with PKH67-stained platelets (green) under static conditions. Images show orthogonal sections of 3D representations. Cell membrane was stained with WGAAlexa 555 (red). Scale bar x: 10 µm; z: 5 µm

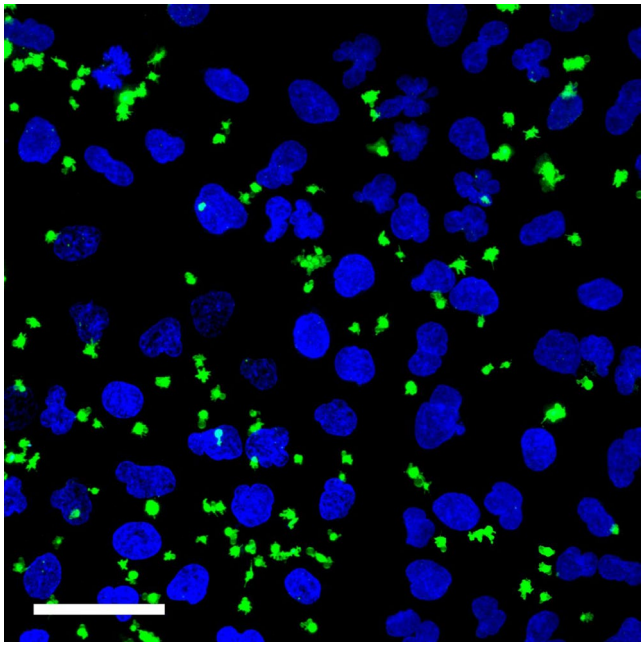

FIGURE 4 Dynamin inhibition blocks platelet uptake in A549 cells confocal laser scanning microscopy image of A549 cells in presence of Dyngo4a after 60 min of incubation with PKH67-stained platelets (green) under static conditions revealed that platelet uptake is impaired by Dyngo4a (30 µm). Nuclei are stained with DAPI (blue). Images are shown as max. Z-projection, scale bar: 50 µm

of tumor growth and metastasis.[34] However, extensive and precise protocols of platelet isolation and handling are rare in these papers. Because our observations are different from what is usually described, details of platelet isolation and handling are given as supporting information (Appendix S1). The cancer cell--platelet interaction we observed is clearly different from the generally accepted concept of an aggregate or cloak formation. Minutes after incubation of cancer cells with fluorescently labeled platelets, the fluorescence signal appeared in the cytosol and plasma membrane of cancer cells. This was shown with labeling the platelet-specific protein CD42a using anti-CD42a-FITC antibodies or the platelet membrane with the membrane-anchoring fluorophore PKH67. Platelets release PMPs *in vivo* under physiological conditions and upon activation. PMPs are 0.1–1-µm fragments and known for their protumorigenic and prometastatic functions.[12] Therefore, PMPs may contribute to the accumulation of platelet fragments in cancer cells.

4.1 | Phagocytotic uptake of platelets by cancer cells

In experiments with PKH67-stained platelets frequently whole platelets penetrating the plasma membrane of A549 were observed (Figure 3 and Movie S1). From this observation it is unlikely that the appearance of intracellular platelet membrane fragments is exclusively attributed to PMPs. Visualization of platelets penetrating the plasma membrane of cancer cells indicated a phagocytic uptake mechanism. Phagocytosis is the uptake pathway for larger particles (300 nm to several µm).[35] Not only specialized cells, like macrophages, but also many other cells are capable of phagocytosis, although to a lesser extent. Also some epithelial cells use phagocytosis for apoptotic cell clearance[23] and regulation of inflammation.[24] We used Dyngo4a, a highly potent inhibitor for Dynamin-1 and Dynamin-2 to test this pathway. Dynamin-2 is a key enzyme for phagocytosis because its inhibition impairs phagocytic cup formation.[30] Although the mechanism of platelet uptake in A549 cancer cells remains unsolved, it turns out that this process is strictly dynamin dependent. This is in good correlation with the observation that the uptake of nanoconjugates and polymeric nanoparticles by cancer cells depends on dynamin function.[36,37] It is noteworthy that Dynamin-2 expression level correlates with tumor invasion, metastasis, poor prognosis, and mortality in several types of cancer.[38] Phagocytosis of platelets by phagocytes in the reticuloendothelial system is a clearance mechanism to maintain normal physiological blood platelet counts. Known platelet clearance mechanisms include Fc receptor– mediated and Fc receptor–independent phagocytosis.[39] To maintain platelets in circulation platelets express CD47 on their surface, which prevents phagocytosis by macrophages. CD47 is recognized by the SIRPα transmembrane protein on phagocytes and transmits anti-phagocytic signals.[40] One Fc receptor–independent pathway is the enhanced exposure of phosphatidylserine to the surface of activated platelets,[41,42] which is recognized by phosphatidylserine receptors on phagocytes like TIM-4 (T-cell immunoglobulin- and mucin-domain-containing molecule 4) and others.[43] It was shown that TIM-4 is expressed in non-small cell lung cancer cells and negatively correlated with survival of patients.[44] This Fc receptor–independent phagocytosis was shown by the uptake of gold particles coated with negatively charged lipids by A549 cells.[45,46]

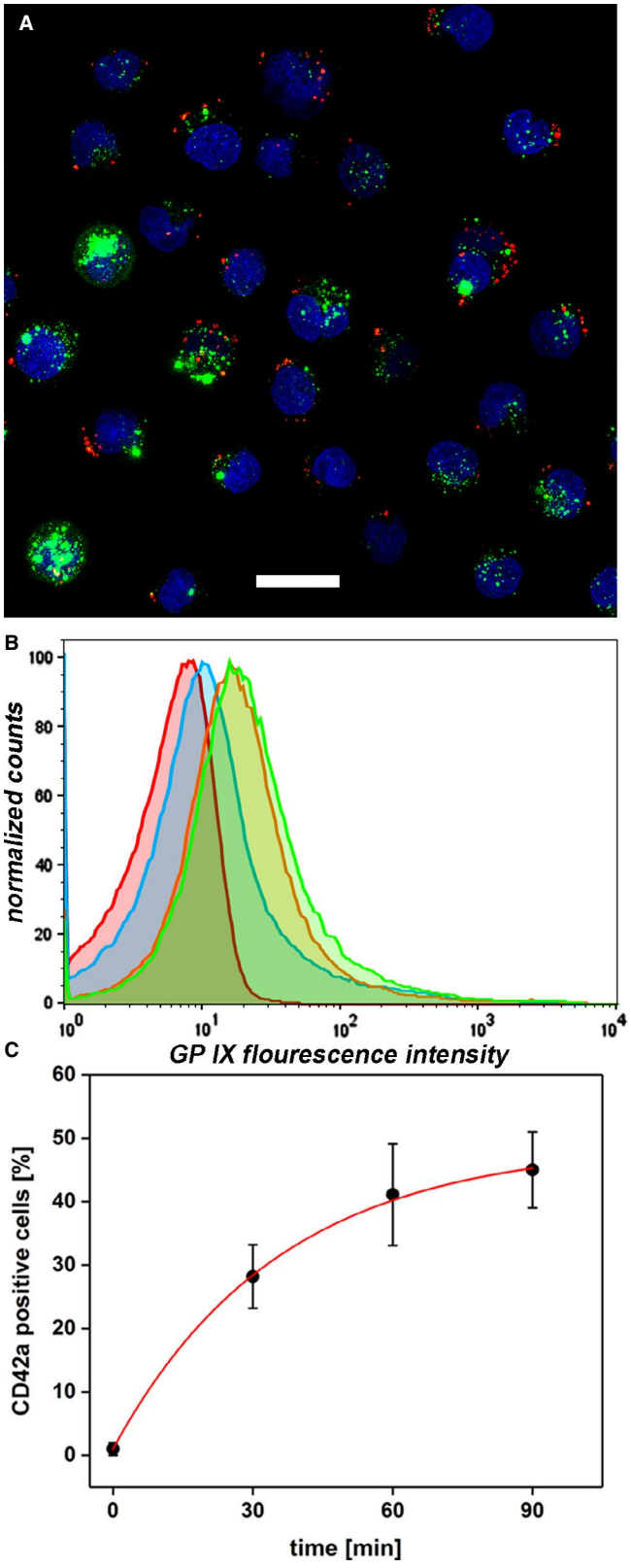

FIGURE 5 Time-dependent appearance of CD42a in A549 plasma membrane. A, Maximum Z-projection of a confocal laser scanning microscopy scan of A549 cells after 90 min of incubation with PKH67-stained platelets (green) under dynamic condition followed by live-cell staining against CD42a (GPIX) using mouse anti-CD42a and anti-mouse-Alexa 555 (red) antibodies. Scale bar: 20 μm. B, Histograms of flow cytometry data of A549 cells incubated with unstained platelets (red) and after 30 (blue), 60 (brown), and 90 (green) min of incubation with CD42a stained platelets. C, Time-response relationship of CD42a appearance on A549 cell membrane. Data follow an exponential decay fit (red line)

A support of our findings about the platelet uptake by cancer cells was published by Sarkar et al.[47] They loaded platelets with the cytostatic drug doxorubicin, added them to A549 cells, and observed cytotoxic response that killed the A549 cells.[47] This was interpreted by a release of the drug in close proximity to the cancer cell upon platelet activation due to the platelet-cancer cell contact. In a later study with drug-loaded PMPs they refined their interpretation to: drug-loaded platelets secrete drug-loaded PMPs upon activation followed by an uptake of these PMPs.[48] Our findings will allow an additional interpretation, namely that not only drug-loaded PMPs but also drug-loaded whole platelets were phagocytosed by A549 cells and the drug was released inside. A strategy of targeted drug delivery to cancer cells is the coating of drug-loaded nanoparticles with platelet membrane.[49–51] These platelet-mimicking nanovehicles show a greater cellular uptake in MDA-MB-231 breast cancer cells and further elicit higher cytotoxicity compared to uncoated nanovehicles.[51] This effect also could be explained in accordance with our findings.

4.2 | Phagocytotic recycling

An interesting feature in Figure 6A is the clear membrane staining in A549 cells. A cell-cell transfer of PKH67 can be excluded because no membrane staining was visible in 16HBE14o$^-$ cells. It is conceivable that platelet membrane was recycled by the cancerous cells upon phagocytosis and incorporated into their own plasma membrane. A potential recycling of platelet proteins was tested by live-cell staining of CD42a on the surface of A549 cells after incubation with PKH67-stained platelets for 30, 60, and 90 min (Figure 5A). FACS analysis revealed an increase of CD42a expression in the membrane of A549 cells over time (Figure 5B and C). The increase of CD42a fluorescence intensity was largest between 30 and 60 min but only small between 60 and 90 min. This correlates with the time course of platelet uptake measured with PKH67 (Figure 2D). Localization of PKH67 fluorescence is assumed intracellularly (Figures 2,3 and Movie S1) whereas CD42a has been found intracellularly (Figure 1A) and extracellularly (Figure 5A). Surprisingly, Figure 6A shows a clear membrane localization of PKH67 fluorescence in A549 cells. Obviously, platelet membrane fragments are also recycled by A549 cells. Because membrane staining was not visible in 16HBE14o$^-$ cells, a cell-cell transfer of PKH67 can be excluded. It seems to be that membrane location of PKH67 correlates with the uptake of PKH67-stained platelets. In Figure 5A two cells with the highest intracellular PKH67 signal show a membrane staining (left side, middle and bottom). Cells in Figure 6A show generally a very high level of PKH67 fluorescence indicating a high level of platelet uptake. Here, membrane localization of PKH67 is clearly visible.

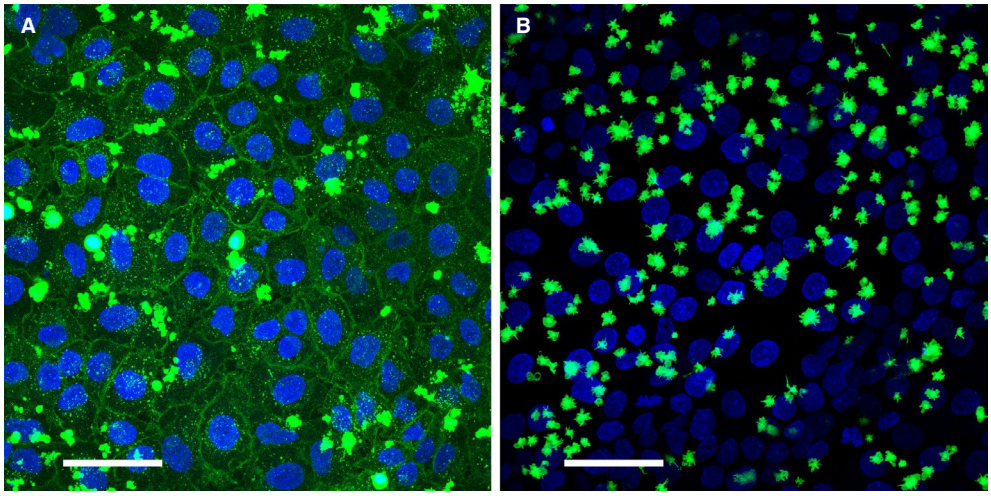

FIGURE 6 Comparison of platelet uptake between cancerous and non-cancerous cells. Incubation with PKH67-stained platelets (green) for 1 h revealed platelet uptake and PKH67 membrane staining in A549 cells (A) but not in 16HBE14o− cells (B). Nuclei are stained with DAPI (blue). Images are shown as max. Z-projection, scale bar: 50 μm

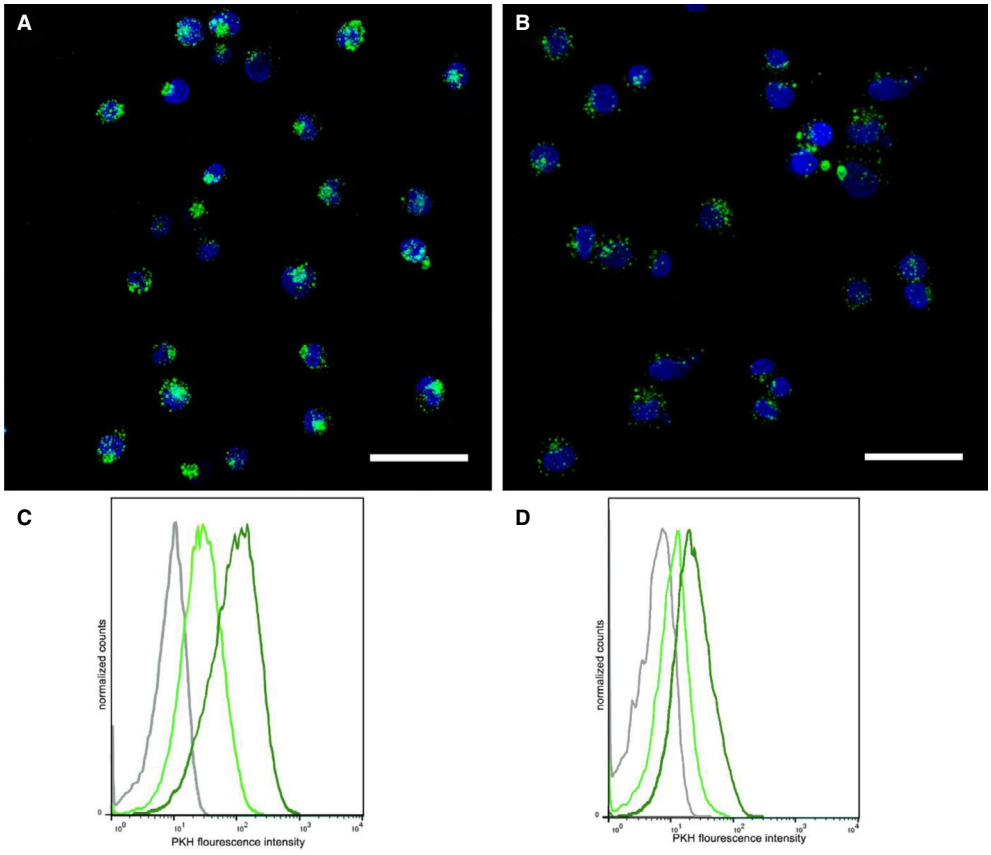

FIGURE 7 Uptake of PKH67-stained platelets in MV3 and MCF-7 cancerous cells. Incubation with PKH67-stained platelets (green) for 90 min under dynamic conditions revealed platelet uptake in MV3 (A) and MCF-7 (B). Nuclei were stained with DAPI (blue). Images are shown as max. Z-projection, scale bar: 50 μm. Flow cytometry analysis showed PKH67 fluorescence intensity from MV3 cells (C) and MCF-7 cells (D) without (gray) and with incubation of PKH67-stained platelets for 30 min (bright green) and 90 min (dark green)

Obviously, the platelet-specific protein CD42a appears in the plasma membrane of A549 cells upon contact with platelets, likely a result of a phagocytic recycling process.[52] The phagosomal cargo is usually subjected to degradation in lysosomes but could also be recycled. Phagocytic recycling follows phagosome formation to recycle plasma membrane and phagocytized proteins by formation

of recycling endosomes and/or via the secretory pathway after retrograde transport from the phagosome to the TGN (for a comprehensive review see Levin et al.[52]). It is likely that cancer cells use this pathway to utilize platelet proteins to, for example, adhere to the vessel wall. CD42a (GPIX) is a part of the von Willebrand factor (VWF) binding complex (GPIb-IX-V complex). It was shown that cancer cells activate endothelial cells (EC) whereupon EC release highly procoagulatory VWF.[53] Tumor cell--induced ultra-large VWF fibers are the preferred target for platelet binding[54] and VWF mediates the adherence of circulating cancer cells to the endothelium[55] via the VWF binding complex. It is noteworthy that several types of cancer are associated with a high plasma level of VWF, for example, prostate cancer, cervical and ovarian carcinoma, head and neck cancer, and colorectal cancer and often correlates with a poor prognosis.[56] Although we show the appearance of only one component of the VWF binding complex in the membrane of A549 cancer cells (CD42a) it is likely that the whole VWF binding complex is recycled: (1) only complete complexes were expressed on the platelet membrane and (2) the absence of one component led to rapid degradation of the VWF binding complex.[57] The GPIb-IX-V complex is also a ligand for endothelial P-selectin,[58] which is upregulated upon endothelial activation.[59] It is well known that shear force is important for normal platelet activation.[60] We have to state that a limitation of this work is the lack of controlled shear stress. Even though we can assume shear forces in the dynamic incubations, it is unlikely that these shear forces met the criteria of shear forces in the bloodstream. However, most published papers about platelet--cancer cell interactions did not apply defined shear stress and shear rates during the incubation of cancer cells with platelets.

The appearance of platelet-specific proteins on the surface of cancer cells was described for MHC class I.[18] This was explained by a transfer of platelet proteins to cancer cells by membrane fusion and transmembraneous integration of these proteins. Obviously, cancer cells use different mechanisms to acquire platelet proteins. Integration of MHC I is important to escape immune surveillance and needs to occur immediately after the cancer cell enters the blood vessel. CD42a exposure is needed for tethering and arrest to the vessel wall and not as time critical as MHC I.

Beside the acquisition of MHC I and CD42a, the uptake of platelets has additional advantages for cancer cells. Platelets contain a diverse transcriptome containing about 9500 mRNAs, a variety of miRNAs, and a repertoire of RNA processing proteins.[14] It was shown that these RNAs significantly change protein expression and signaling pathways related to survival and proliferation in cancer cells after coincubation with platelets.[61] An example is miR-223, which is delivered by platelet-derived particles and was found to promote A549 cell invasion via targeting of tumor suppressor EPB41L3.[62] There is also evidence that cancer cells can exhibit a "platelet mimicry," by expressing platelet-specific proteins such as GPIIb-IIIa, protease-activated receptors (PARs), and platelet endothelial cell adhesion molecule 1 (PECAM1).[63] It is generally assumed that the transfer of RNA is mediated by PMPs[14] and exosomes.[5] Our results complement this aspect by uptake of whole platelets as another way of RNA transfer.

4.3 | Platelet uptake by different cell lines

The results obtained with A549 cells leads to the question if the uptake of platelets is specific for A549 cells or a more general mechanism by which cancer cells hijack the service of platelets. In a first step toward an answer we used the melanoma-derived highly metastatic cell line MV3;[32] the mammary ductal carcinoma cell line MCF-7;[33] and the non-cancerous, SV40 large T-antigen transformed, lung epithelial cell line (16HBE14o$^-$).[31] Incubation with PKH67-stained platelets for 1 h revealed an uptake of platelets in all cancer cell lines (Figure 6A and 7) but not in the non-cancerous 16HBE14o$^-$ cells (Figure 6B). No platelet uptake but activated platelets were found spread on the membrane of 16HBE14o$^-$ (Figure 6B). This indicated that platelets show an initial but not a full activation[64] upon contact with 16HBE14o$^-$ cells. However, phagocytosis did not occur. In general, platelet activation is a term to describe the multi-step behavior of platelets upon agonist stimulation. This includes shape change, exposure of aminophospholipids, secretion of granules, microparticle formation, and aggregation. Here, platelet shape change is visible indicating at least partially activated platelets. The appearance of spread-out platelets on A549 cells (Figure 4) indicates that (partial) activation of platelets occurs also on cancerous cells. These results show that platelet uptake is not a specific characteristic of A549 cells but it can be assumed as a property of cancerous cells. Platelet uptake studies with several cancerous and non-cancerous cells are necessary to test this assumption.

Together, this work revealed that (1) A549 cancer cells phagocytose platelets and utilize platelet proteins through phagocytic recycling to their cell surface. This was shown for CD42a, a constituent of the VWF binding complex. (2) Platelet uptake was shown for cancerous lung epithelial cells (A549) whereas non-cancerous lung epithelial cells (16HBE14o$^-$) show no platelet uptake. (3) All cancer cell lines tested in this work show a platelet uptake. Beside the utilization of CD42a, the uptake of platelets has additional advantages for cancer cells: Access to the entire platelet content, including their complete and undiluted proteome,[65] transcriptome,[66] and secretome.[67] It is likely that the cancer cells exploit all these constituents of the platelet to promote its survival and metastatic potential. Up to now platelet-derived microparticles and exosomes were suspected to mediate changes in the tumor cell and promote metastasis. This would allow cancer cells to exploit most of the platelets' contents but phagocytosis of whole platelets increases the amount and uptake rate of platelets' proteome, transcriptome, and secretome.

This new aspect of platelet-cancer cell interaction shown in this work has various consequences for therapy and diagnosis.

4.3.1 | Testing antiplatelet drugs

Inhibiting the tumor--platelet cross-talk is a promising tumor treatment strategy and antiplatelet therapy has become a standard intervention of cancer in the past.[68] Nevertheless, there is a need for more effective and non-toxic antiplatelet agents that will not increase bleeding risk.[69] Our approach represents a simple and reliable model to test the efficiency of antiplatelet drugs to inhibit platelet-tumor cell interaction. In addition, this platelet uptake assay could be easily automated.

4.3.2 | Platelet-based drug delivery systems

The observation of platelet uptake by cancer cells could spur the development of new platelet-based tumor-targeted drug delivery systems. Using platelets as tumor-targeted drug delivery is of increasing importance in antitumor therapy (reviewed in Du et al.[70]) because drug-loaded platelets reduce systemic toxicity, and enhance targeting and the penetrability of drugs to tumor cells. Our findings complement existing knowledge and may help to optimize strategies for further platelet-based drug delivery systems not only for classical chemotherapy agents but also for the new generation of RNA-based anti-cancer drugs.

4.3.3 | Diagnosis

Discovery of tumor-educated platelets (TEPs) increased the knowledge about metastasis and led to a series of new diagnostic approaches.[71] In analogy to TEPs cancer cells that have ingested platelets could be named platelet-educated tumor cells (PETCs). We observed the difference in platelet uptake between healthy and cancerous lung epithelial cells (Figure 6). Potentially, these PETCs could allow determining the metastatic potential of a tumor cell by its platelet uptake rate. However, platelet uptake studies with low- and high metastatic cell types from the same origin are necessary to shed light on this idea.

5 | CONCLUSION

The phagocytic uptake of platelets by cancer cells and recycling of CD42a shown in this work reveals a novel aspect of platelet--cancer cell interactions by which platelets unfold their protumorigenic and prometastatic effects.

These new insights in platelet-cancer cell interactions will allow further mechanistic elucidation of platelet-assisted metastasis and thus may contribute to the optimization of cancer therapies.

ACKNOWLEDGMENTS

We thank Dr. Jana Zimmermann and Dr. Raquel Sanz-Soler from the Institute of Biochemistry and Pathophysiology and thanks also to Dr. Dieter Gruenert (formerly of the University of California, San Francisco, USA) for the generous gift of the 16HBE14o– cell line. This work was financially supported by Cells-in-Motion Cluster of Excellence EXC 1003–CiM (University of Muenster) (HS) and the doctoral training program MedK Muenster (NMC, AS, SB). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

N. Martins Castanheira, A. Spanhofer, and S. Wiener performed the experiments; data analysis and interpretation were done by N. Martins Castanheira, A. Spanhofer, S. Wiener, and S. Bobe; H. Schillers designed the study, supervised the analysis, and wrote the manuscript. All authors contributed to manuscript revision, and read and approved the submitted version.

ORCID

Sebastian Wiener https://orcid.org/0000-0002-3119-7876
Hermann Schillers https://orcid.org/0000-0001-6090-4771

REFERENCES

1. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. *Nat Med*. 2006;12:895-904. doi:10.1038/nm1469
2. Li N. Platelets in cancer metastasis: to help the "villain" to do evil. *Int J Cancer*. 2016;138:2078-2087. doi:10.1002/ijc.29847
3. Bastida E, Almirall L, Ordinas A. Tumor-cell-induced platelet aggregation is a glycoprotein-dependent and lipoxygenase-associated process. *Int J Cancer*. 1987;39:760-763.
4. van Es N, Sturk A, Middeldorp S, Nieuwland R. Effects of cancer on platelets. *Semin Oncol*. 2014;41:311-318. doi:10.1053/j.seminoncol.2014.04.015
5. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. *Nat Rev Cancer*. 2011;11:123-134. doi:10.1038/nrc3004
6. Palumbo JS, Talmage KE, Massari JV, et al. Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood*. 2005;105:178-185. doi:10.1182/blood-2004-06-2272
7. Nieswandt B, Hafner M, Echtenacher B, Mannel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Can Res*. 1999;59:1295-1300.
8. Menter DG, Kopetz S, Hawk E, et al. Platelet "first responders" in wound response, cancer, and metastasis. *Cancer Metastasis Rev*. 2017;36:199-213. doi:10.1007/s10555-017-9682-0
9. Dovizio M, Ballerini P, Fullone R, Tacconelli S, Contursi A, Patrignani P. Multifaceted functions of platelets in cancer: from tumorigenesis to liquid biopsy tool and drug delivery system. *Int J Mol Sci*. 2020;21:9585. doi:10.3390/ijms21249585
10. Best MG, Sol N, Kooi I, et al. RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. *Cancer Cell*. 2015;28:666-676. doi:10.1016/j.ccell.2015.09.018
11. Best MG, Wesseling P, Wurdinger T. Tumor-educated platelets as a noninvasive biomarker source for cancer detection and progression monitoring. *Can Res*. 2018;78:3407-3412. doi:10.1158/0008-5472.CAN-18-0887

12. Burnouf T, Goubran HA, Chou ML, Devos D, Radosevic M. Platelet microparticles: detection and assessment of their paradoxical functional roles in disease and regenerative medicine. *Blood Rev*. 2014;28:155-166. doi:10.1016/j.blre.2014.04.002
13. Siljander PR. Platelet-derived microparticles - an updated perspective. *Thromb Res*. 2011;127(Suppl 2):S30-S33. doi:10.1016/S0049-3848(10)70152-3
14. Provost P. The clinical significance of platelet microparticle-associated microRNAs. *Clin Chem Lab Med*. 2017;55:657-666. doi:10.1515/cclm-2016-0895
15. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood*. 1999;94:3791-3799.
16. Mittelbrunn M, Sanchez-Madrid F. Intercellular communication: diverse structures for exchange of genetic information. *Nat Rev Mol Cell Biol*. 2012;13:328-335. doi:10.1038/nrm3335
17. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell*. 2011;20:576-590. doi:10.1016/j.ccr.2011.09.009
18. Sol N, Wurdinger T. Platelet RNA signatures for the detection of cancer. *Cancer Metastasis Rev*. 2017;36:263-272. doi:10.1007/s10555-017-9674-0
19. Kumari S, Chaurasia SN, Nayak MK, Mallick RL, Dash D. Sirtuin inhibition induces apoptosis-like changes in platelets and thrombocytopenia. *J Biol Chem*. 2015;290:12290-12299. doi:10.1074/jbc.M114.615948
20. Maugeri N, Rovere-Querini P, Evangelista V, et al. Neutrophils phagocytose activated platelets in vivo: a phosphatidylserine, P-selectin, and {beta}2 integrin-dependent cell clearance program. *Blood*. 2009;113:5254-5265. doi:10.1182/blood-2008-09-180794
21. Kirschbaum M, Karimian G, Adelmeijer J, Giepmans BNG, Porte RJ, Lisman T. Horizontal RNA transfer mediates platelet-induced hepatocyte proliferation. *Blood*. 2015;126:798-806. doi:10.1182/blood-2014-09-600312
22. Peng B, Koga K, Cardenas I, Aldo P, Mor G. Phagocytosis of apoptotic trophoblast cells by human endometrial endothelial cells induces proinflammatory cytokine production. *Am J Reprod Immunol*. 2010;64:12-19. doi:10.1111/j.1600-0897.2010.00815.x
23. Guo F, Ding Y, Caberoy N, et al. ABCF1 extrinsically regulates retinal pigment epithelial cell phagocytosis. *Mol Biol Cell*. 2015;26:2311-2320. doi:10.1091/mbc.E14-09-1343
24. Monks J, Rosner D, Jon Geske F, et al. Epithelial cells as phagocytes: apoptotic epithelial cells are engulfed by mammary alveolar epithelial cells and repress inflammatory mediator release. *Cell Death Differ*. 2005;12:107-114. doi:10.1038/sj.cdd.4401517
25. Jiang P, Ren YL, Lan Y, et al. Phagocytosis of platelets enhances endothelial cell survival under serum deprivation. *Exp Biol Med*. 2015;240:876-883. doi:10.1177/1535370214565076
26. Gu R, Sun X, Chi Y, et al. Integrin beta3/Akt signaling contributes to platelet-induced hemangioendothelioma growth. *Sci Rep*. 2017;7:6455. doi:10.1038/s41598-017-06927-0
27. Liebsch AG, Schillers H. Quantification of heparin's antimetastatic effect by single-cell force spectroscopy. *J Mol Recognit*. 2021;34:e2854. doi:10.1002/jmr.2854
28. Haka AS, Grosheva I, Chiang E, et al. Macrophages create an acidic extracellular hydrolytic compartment to digest aggregated lipoproteins. *Mol Biol Cell*. 2009;20:4932-4940. doi:10.1091/mbc.E09-07-0559
29. Motskin M, Muller KH, Genoud C, Monteith AG, Skepper JN. The sequestration of hydroxyapatite nanoparticles by human monocyte-macrophages in a compartment that allows free diffusion with the extracellular environment. *Biomaterials*. 2011;32:9470-9482. doi:10.1016/j.biomaterials.2011.08.060
30. Marie-Anais F, Mazzolini J, Herit F, Niedergang F. Dynamin-actin cross talk contributes to phagosome formation and closure. *Traffic*. 2016;17:487-499. doi:10.1111/tra.12386
31. Cozens AL, Yezzi MJ, Kunzelmann K, et al. CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol*. 1994;10:38-47.
32. van Muijen GN, Jansen KFJ, Cornelissen IMHA, Smeets DFCM, Beck JLM, Ruiter DJ. Establishment and characterization of a human melanoma cell line (MV3) which is highly metastatic in nude mice. *Int J Cancer*. 1991;48:85-91. doi:10.1002/ijc.2910480116
33. Ziegler E, Hansen MT, Haase M, Emons G, Grundker C. Generation of MCF-7 cells with aggressive metastatic potential in vitro and in vivo. *Breast Cancer Res Treat*. 2014;148:269-277. doi:10.1007/s10549-014-3159-4
34. Plantureux L, Mège D, Crescence L, et al. The interaction of platelets with colorectal cancer cells inhibits tumor growth but promotes metastasis. *Can Res*. 2020;80:291-303. doi:10.1158/0008-5472.CAN-19-1181
35. Morrissette N, Gold E, Aderem A. The macrophage–a cell for all seasons. *Trends Cell Biol*. 1999;9:199-201.
36. Gundel D, Allmeroth M, Reime S, Zentel R, Thews O. Endocytotic uptake of HPMA-based polymers by different cancer cells: impact of extracellular acidosis and hypoxia. *Int J Nanomed*. 2017;12:5571-5584. doi:10.2147/IJN.S136952
37. Li F, Zhao Y, Mao C, Kong Y, Ming X. RGD-modified albumin nanoconjugates for targeted delivery of a porphyrin photosensitizer. *Mol Pharm*. 2017;14:2793-2804. doi:10.1021/acs.molpharmaceut.7b00321
38. Meng J. Distinct functions of dynamin isoforms in tumorigenesis and their potential as therapeutic targets in cancer. *Oncotarget*. 2017;8:41701-41716. doi:10.18632/oncotarget.16678
39. Grozovsky R, Hoffmeister KM, Falet H. Novel clearance mechanisms of platelets. *Curr Opin Hematol*. 2010;17:585-589. doi:10.1097/MOH.0b013e32833e7561
40. Olsson M, Bruhns P, Frazier WA, Ravetch JV, Oldenborg PA. Platelet homeostasis is regulated by platelet expression of CD47 under normal conditions and in passive immune thrombocytopenia. *Blood*. 2005;105:3577-3582. doi:10.1182/blood-2004-08-2980
41. Halliez M, Fouassier M, Robillard N, et al. Detection of phosphatidyl serine on activated platelets' surface by flow cytometry in whole blood: a simpler test for the diagnosis of Scott syndrome. *Br J Haematol*. 2015;171(2):290-292. doi:10.1111/bjh.13391
42. Dachary-Prigent J, Freyssinet JM, Pasquet JM, Carron JC, Nurden AT. Annexin V as a probe of aminophospholipid exposure and platelet membrane vesiculation: a flow cytometry study showing a role for free sulfhydryl groups. *Blood*. 1993;81:2554-2565.
43. Toda S, Nishi C, Yanagihashi Y, Segawa K, Nagata S. Clearance of apoptotic cells and pyrenocytes. *Curr Top Dev Biol*. 2015;114:267-295. doi:10.1016/bs.ctdb.2015.07.017
44. Zhang Q, Wang H, Wu X, et al. TIM-4 promotes the growth of non-small-cell lung cancer in a RGD motif-dependent manner. *Br J Cancer*. 2015;113:1484-1492. doi:10.1038/bjc.2015.323
45. Wang M, Petersen NO. Lipid-coated gold nanoparticles promote lamellar body formation in A549 cells. *Biochim Biophys Acta*. 2013;1831(6):1089-1097. doi:10.1016/j.bbalip.2013.01.018
46. Albrecht S, Usmani SM, Dietl P, Wittekindt OH. Plasma membrane trafficking in alveolar type II cells. *Cell Physiol Biochem*. 2010;25:81-90. doi:10.1159/000272053
47. Sarkar S, Alam MA, Shaw J, Dasgupta AK. Drug delivery using platelet cancer cell interaction. *Pharm Res*. 2013;30:2785-2794. doi:10.1007/s11095-013-1097-1
48. Sarkar S, Dasgupta AK. Microparticle of drug and nanoparticle: a biosynthetic route. *Pharmacol Res Perspect*. 2015;3:e00188. doi:10.1002/prp2.188
49. Wang H, Wu J, Williams GR, et al. Platelet-membrane-biomimetic nanoparticles for targeted antitumor drug delivery. *J Nanobiotechnology*. 2019;17:60. doi:10.1186/s12951-019-0494-y

50. Hu Q, Sun W, Qian C, Wang C, Bomba HN, Gu Z. Anticancer platelet-mimicking nanovehicles. *Adv Mater*. 2015;27:7043-7050. doi:10.1002/adma.201503323
51. Ye H, Wang K, Wang M, et al. Bioinspired nanoplatelets for chemo-photothermal therapy of breast cancer metastasis inhibition. *Biomaterials*. 2019;206:1-12. doi:10.1016/j.biomaterials.2019.03.024
52. Levin R, Grinstein S, Canton J. The life cycle of phagosomes: formation, maturation, and resolution. *Immunol Rev*. 2016;273:156-179. doi:10.1111/imr.12439
53. Bauer AT, Suckau J, Frank K, et al. von Willebrand factor fibers promote cancer-associated platelet aggregation in malignant melanoma of mice and humans. *Blood*. 2015;125:3153-3163. doi:10.1182/blood-2014-08-595686
54. Schneider SW, Nuschele S, Wixforth A, et al. Shear-induced unfolding triggers adhesion of von Willebrand factor fibers. *Proc Natl Acad Sci USA*. 2007;104:7899-7903. doi:10.1073/pnas.0608422104
55. Morganti M, Carpi A, Amo-Takyi B, et al. Von Willebrand's factor mediates the adherence of human tumoral cells to human endothelial cells and ticlopidine interferes with this effect. *Biomed Pharmacother*. 2000;54:431-436.
56. Wang WS, Lin JK, Lin TC, et al. Plasma von Willebrand factor level as a prognostic indicator of patients with metastatic colorectal carcinoma. *World J Gastroenterol*. 2005;11:2166-2170.
57. Dong JF, Gao S, Lopez JA. Synthesis, assembly, and intracellular transport of the platelet glycoprotein Ib-IX-V complex. *J Biol Chem*. 1998;273:31449-31454.
58. Romo GM, Dong JF, Schade AJ, et al. The glycoprotein Ib-IX-V complex is a platelet counterreceptor for P-selectin. *J Exp Med*. 1999;190:803-814.
59. Gotsch U, Jager U, Dominis M, Vestweber D. Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-alpha in vivo. *Cell Adhes Commun*. 1994;2:7-14.
60. Pang A, Cui Y, Chen Y, et al. Shear-induced integrin signaling in platelet phosphatidylserine exposure, microvesicle release, and coagulation. *Blood*. 2018;132:533-543. doi:10.1182/blood-2017-05-785253
61. Haemmerle M, Taylor ML, Gutschner T, et al. Platelets reduce anoikis and promote metastasis by activating YAP1 signaling. *Nat Commun*. 2017;8:310. doi:10.1038/s41467-017-00411-z
62. Liang H, Yan X, Pan Yi, et al. MicroRNA-223 delivered by platelet-derived microvesicles promotes lung cancer cell invasion via targeting tumor suppressor EPB41L3. *Mol Cancer*. 2015;14:58. doi:10.1186/s12943-015-0327-z
63. Bambace NM, Holmes CE. The platelet contribution to cancer progression. *J Thromb Haemost*. 2011;9:237-249. doi:10.1111/j.1538-7836.2010.04131.x
64. Tomaiuolo M, Brass LF, Stalker TJ. Regulation of platelet activation and coagulation and its role in vascular injury and arterial thrombosis. *Interv Cardiol Clin*. 2017;6:1-12. doi:10.1016/j.iccl.2016.08.001
65. Burkhart JM, Gambaryan S, Watson SP, et al. What can proteomics tell us about platelets? *Circ Res*. 2014;114:1204-1219. doi:10.1161/CIRCRESAHA.114.301598
66. Nassa G, Giurato G, Cimmino G, et al. Splicing of platelet resident pre-mRNAs upon activation by physiological stimuli results in functionally relevant proteome modifications. *Sci Rep*. 2018;8:498. doi:10.1038/s41598-017-18985-5
67. Golebiewska EM, Poole AW. Platelet secretion: from haemostasis to wound healing and beyond. *Blood Rev*. 2015;29:153-162. doi:10.1016/j.blre.2014.10.003
68. Tao DL, Tassi Yunga S, Williams CD, McCarty OJT. Aspirin and antiplatelet treatments in cancer. *Blood*. 2021;137:3201-3211. doi:10.1182/blood.2019003977
69. Lopez JA. Introduction to a review series on platelets and cancer. *Blood*. 2021;137:3151-3152. doi:10.1182/blood.2020010237
70. Du Y, Wang S, Zhang M, Chen B, Shen Y. Cells-based drug delivery for cancer applications. *Nanoscale Res Lett*. 2021;16:139. doi:10.1186/s11671-021-03588-x
71. Roweth HG, Battinelli EM. Lessons to learn from tumor-educated platelets. *Blood*. 2021;137:3174-3180. doi:10.1182/blood.2019003976

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Martins Castanheira N, Spanhofer AK, Wiener S, Bobe S, Schillers H. Uptake of platelets by cancer cells and recycling of the platelet protein CD42a. *J Thromb Haemost*. 2021;00:1–12. doi:10.1111/jth.15543